JOURNAL

OF

HELMINTHOLOGY

(Founded by R. T. LEIPER)

Vol. XXXV, 1961

Edited by

J. J. C. BUCKLEY, D.Sc.
William Julien Courtauld Professor of Helminthology
in the University of London

London School of Hygiene
and
Tropical Medicine
Keppel Street
London
W.C.1

CONTENTS OF VOLUME XXXV

Nos. 1/2

		PAGES
1.	Ali, S. M. On Some New Nematodes (Habronematinae) from Birds in Hyderabad, India, and the Relationships of the Genus *Habronema*	1—48
2.	Ali, S. M. On Some New Species of *Physaloptera* from Indian Birds	49—60
3.	Bernard, J. On Two Capillariid Nematodes Parasitic in British Water Shrews (*Neomys fodiens* Schr.)	61—68
4.	Dinnik, J. A. *Paramphistomum phillerouxi* sp. nov. (Trematoda : Paramphistomatidae) and its Development in *Bulinus forskalii*	69—90
5.	Fitzsimmons, W. M. Redescription of *Labiduris africana* Gedoelst, 1916 with New Host Records and a Summary of our Present Knowledge of of the Genus *Labiduris* Schneider, 1866	91—100
6.	Gharib, H. M. On the Migration Route of the Infective Larvae of *Nippostrongylus brasiliensis* ...	101—108
7.	Gharib, H. M. Some Observations on the Behaviour of *Nippostrongylus brasiliensis* in Different Kinds of Laboratory Animals	109—114
8.	Honer, M. R. and Jansen, J. On *Daubaylia elegans* n.sp., in *Planorbis carinatus* Müller	115—118
9.	Khan, D. The Life History of *Echinostoma londonensis* n.sp., the Adult of *Cercaria londonensis* Khan, 1960	119—132
10.	Khan, D. Studies on Larval Trematodes Infecting Freshwater Snails in London (U.K.) and Some Adjoining Areas. Part III. "Lophocercous" Cercariae	133—142

PAGES

11. Nelson, G. S. On *Dipetalonema manson-bahri* n.sp., from the Spring-hare, *Pedetes surdaster larvalis*, with a Note on its Development in Fleas ... 143—160

12. Varma, A. K. Observations on the Biology and Pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901) ... ... 161—168

13. Wahid, S. Systematic Studies on Some Cestodes of Reptiles and Birds ... ... 169—180

14. Williams, J. B. The Dimorphism of *Polystoma integerrimum* (Frölich) Rudolphi and Its Bearing on Relationships within the Polystomatidae : Part III ... ... 181—202

15. Yamaguti, S. *Oesophagostomum watanabei* n.sp. (Nematoda, Strongylidae) from a Japanese Wild Boar ... ... 203—206

Nos. 3/4

1. Desowitz, R. S., Wong, H. J. and Fernando, M. A. The First Record of Human Infection with *Bertiella studeri* in Malaya ... ... 207—208

2. Dissanaike, A. S. and Paramananthan, D. C. On *Brugia* (*Brugiella* subgen. nov.) *buckleyi* n.sp., from the Heart Blood and Vessels of the Ceylon Hare ... ... 209—220

3. Dissanaike, A. S. and Paramananthan, D. C. On an Unusual Brood Capsule from a Hydatid Cyst of a Goat in Ceylon ... ... 221—224

4. Gharib, H. M. A Preliminary Note on the Bacteriostatic Properties of Some Helminths of Animals 225—232

5. Hockley, A. R. On *Skrjabinotaenia cricetomydis* n.sp. (Cestoda : Anoplocephalata) from the Gambian Pouched Rat ... ... 233—254

6. Khalil, L. F. On a New Cestode, *Paradilepis maleki* sp. nov. (Diplepididae), from a Sacred Ibis in the Sudan ... ... 255—258

7. Khalil, L. F. On a New Trematode, *Orientocreadium lazeri* sp. nov., from a Freshwater Fish, *Clarias lazera*, in the Sudan ... ... 259—262

PAGES

8. Khalil, L. F. On a New Nematode, *Rhaphidascaroides* sp. nov., from a Freshwater Fish, *Gymnarchus niloticus*, in the Sudan ... ... 263—268

9. Khalil, L. F. On the Capture and Destruction of Miracidia by *Chaetogaster limnaei* (Oligochaeta) 269—274

10. Khan, D. Studies on Larval Trematodes Infecting Freshwater Snails in London (U.K.) and Some Adjoining Areas. Part IV. Schistosomatid Cercariae ... ... 275—284

11. Laurence, B. R. and Pester, F. R. N. The Behaviour and Development of *Brugia patei* (Buckley, Nelson and Heisch, 1958) in a Mosquito Host, *Mansonia uniformis* (Theobald) ... ... 285—300

12. Rao, K. H. *Elongoparorchis pneumatis* gen. et sp. nov. (Trematoda) from the Air-bladder of the Marine Catfish, *Arius jella* (Val.)... ... ... 301—304

13. Sandars, D. F. Pulmoverminae n.subfam. (Trematoda : Digenea) for the Genera *Pulmovermis* and *Hydrophitrema* ... ... 305—308

14. Sturrock, R. F. The Quantitative Use of the Seinhorst "Mistifier" to Recover Nematodes from Soil, Faeces and Herbage... ... ... 309—314

15. Taffs, L. F. The Passive Transfer of Immunity in Guineapigs Infected with the Nematode *Ascaris suum* Goeze, 1782 ... ... 315—318

16. Taffs, L. F. Immunological Studies on Experimental Infection of Pigs with *Ascaris suum* Goeze, 1782. I. An Introduction with a Review of the Literature and the Demonstration of Complement-fixing Antibodies in the Serum. ... ... 319—344

17. Wahid, S. On Two New Species of the Genus *Enterobius* Leach, 1853, from a Colobus Monkey 345—352

18. Wahid, S. On *Amplicaecum iguanae* n.sp. with Notes on *Hexametra sewelli, Kalicephalus micrurus* and *Viguiera euryoptera* ... ... 353—360

Index to Volume XXXV ... ... 361—367

Actual Dates of Publication, Volume XXXIV ... 367

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, pp. 1–48.

On Some New Nematodes (Habronematinae) from Birds in Hyderabad, India, and the Relationships of the Genus *Habronema*

By Syed Mehdi Ali, Ph.D., M.Sc.
Reader in Zoology, Osmania University Hyderabad, India
From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The genus *Habronema* has had a very chequered history. Its taxonomy and the relationship of various genera associated with it, have long been a matter of controversy. An attempt is made in this paper to accumulate as far as possible, the facts pertaining to the genus and its relationship with others.

Filaria muscae was the first of this group and was described in 1861 by Carter, as a parasite of the horse, but in the same year Diesing transferred it from "filarids" to spirurids, renaming it *Habronema muscae*. Since then more than forty species have been added to the group ; several species have been removed from *Habronema* to other genera and *vice versa*. Several worms described earlier as "*Filaria*", "*Spiroptera*" and "*Spirura*" have been renamed *Habronema*.

Ransom (1911) brought Schneider's (1866) *Filaria microstoma* into this group, as *Habronema microstoma*. Seurat transferred seven species to this genus as follows : *H. chevreuxi* 1913; *H. leptopterum* 1914 ; *H. mansioni* 1914 ; *H. unilateralis* 1914 ; *H. nouveli* 1915 ; *H. grimaldiae* 1915 and *H. ficheuri* 1916. Of these, three (the first, fifth and sixth) are reported from mammals and the rest are from avian hosts. In 1917 Skrjabin paid tribute to Seurat by naming his species *H. seurati*, the host being a bird of prey, a falcon. In 1918 Lopez-Neyra transferred this worm to *Cyrnea* Seurat, 1914, a genus very closely allied to *Habronema*. Theiler (1923) reported another parasite of this group from the African Zebra, *H. zebrae*. In 1922 and 1923 Gendre made a notable contribution by adding five more species to the genus, namely, *H. longistriata* 1922 ; *H. incertum* 1922 ; *H. tulostoma* 1922, *H. spinosum* 1923 and *H. monopterum* 1923. The first three species were described earlier by Molin (1859), Smith (1906) and Hemprich and Ehrenberg, (1866), respectively as *Spiroptera*, the other two were new species. Walton (1923) contributed another species, *H. colaptes* and in 1928 in revising the nematodes of the

Leidy collection in America, he found that the worm previously described by Molin (1860) as *Spiroptera*, by Diesing (1861) as *Cheilospirura* and by Railliet and Henry (1911) as *Spirura*, was in fact *Habronema* and was named by him *H. uncinipenis*. He also reported two more new species, *H. pileata* and *H. longispiculum*. All three are parasites of birds.

Popowa (1927), reported *H. dipterum* from *Upupa epops*, a bird having a wide distribution in Afro-Eurasian countries. The same parasite was later collected from the same host in China by Faust. Li (1934) studied this material and found that the worm did not agree in certain essential characters with *Habronema*, so he erected a new genus, *Sicarius*, for its reception. Li also reported a new species *H. circi* along with *H. mansioni* and *H. imbricatum* Maplestone, 1930. Baylis 1931 renamed *Protospirura hamospiculata* Neveu-Lemaire, 1927 as *Habronema hamospiculatum*. He also renamed in 1938 *Filaria zschokkei* Meyer, 1896, as *H. zschokkei*. Chabaud (1959) synonymised it with *Gendrespirura hamospiculum*. Maplestone reporting on "the parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens", added nine more new species to the existing dozen or more species. In 1929 *H. indicum* was reported from the Indian roller, *Coracias benghalensis*. In 1930 two species were described : *H. euplocami* from a kalij pheasant and a peacock pheasant, and *H. imbricatum* from a scops owl (*Otus sunia*). In the subsequent year Maplestone added some more species to his list, these being *H. diesingi*, *H. magnilabiatum*, *H. asymmetricum* and *H. casuarii*. Wehr described *H. urophasiana* from the sage grouse in America in 1931.

In 1934 and 1938 two more species, *H. murrayi* and *H. numidica* were reported by Ortlepp from Africa. In 1936 and 1937 two more species from mammalian hosts were added to the genus : *H. manidis* by Vuylsteke and *H. clarki* by Foster and Chitwood. In 1937 however Vuylsteke changed the name of her species to *H. congolense*. Chabaud (1959) correctly regards it as *Gendrespirura hamospiculata*. Johnston and Mawson (1941) also contributed two species, *H. aegotheles* and *H. paraleptoptera* from Australian birds of prey and in the same year (1941) Chandler reported *H. americanum* from *Buteo platypterus*. In 1945, Ezzat Abd Elmoneim reported yet another species, *H. khalili* from Egypt, the host being *Rhinoceros bicornis*. Freitas and Lent (1947) contributed *H. waltoni* from an avian host. Vuylsteke (1953) redescribed *H. skrjabini* reported earlier by Tschernikowa in 1934. *H. buckleyi* was described by Bisseru in 1955 from another avian host, a bird of prey. Chabaud and Brygoo added

the latest species to the group in 1958 by describing *Cyrnea* (*Procyrnea*) *dolichocolpos* from Accipitriform birds in Madagascar.

Meanwhile the disintegration of *Habronema* had already started. Baylis and Daubney indicated in 1926 that *Cyrnea* should be regarded as a synonym of *Habronema*, but in the same year (1926), Yorke and Maplestone accepted the two genera as distinct and valid and this was supported by Cram (1927). In 1930 Maplestone changed his earlier opinion and stated,

> "That Baylis and Daubney are correct in making *Cyrnea* a synonym of *Habronema*, and that any attempt to distinguish between them, in the light of present knowledge, is more likely to lead to confusion than to simplification."

In 1934, Baylis erected a new genus *Cyathospirura* for *H. chevreuxi* and transferred another two species, *H. grimaldiae* and *H. nouveli* to it, all the three species being parasites of carnivores. Strand (1929) erected a new genus, *Seurocyrnea* for *Cyrnea* Seurat, 1914. His genus was accepted by Chitwood and Wehr and incorporated into Habronematinae (1932) but Chabaud (1958) refutes the validity of this genus, on the basis that Strand confused *Cyrnea* for *Cyrena*—a genus erected by Lamark—as already occupied. Hence Chabaud declared *Seurocyrnea* Strand, 1929 as invalid and revived *Cyrnea* again.

Chitwood and Wehr in their monumental work, "The value of cephalic structures as characters in nematode classification", accepted *Habronema muscae* Diesing, 1861, as the type species of genus *Habronema* and *Cyrnea eurycerca* Seurat, 1914, as the type species of *Seurocyrnea*. Neither in their earlier paper (1932) nor in the latter did they mention the reasons for the transference of the avian species of *Habronema* to *Seurocynea*, but in their latter paper, in Table I, they indicated *Habronema* as having mammals as their hosts and *Seurocyrnea* as parasites of birds. Thus, by implication they transferred all the species of *Habronema* having avian hosts to *Seurocyrnea*. Evidently the basis for this transfer seems to be host-parasite specificity rather than morphology. The only morphological difference between the two groups, according to them, seems to be the pre- and the post-equatorial position of the vulva.

Osche redescribed *Habronema mansioni* in 1955 and gave his version of the cephalic end and its constituent parts.

In 1958 Chabaud published his "Essai de Classification des nematodes Habronematinae." He based his thesis on the assumption

that the sub-family Habronematinae has evolved from a hypothetical protospirurid. He has accepted to a certain extent arrangements of the cephalic papillae as described by Chitwood and Wehr and also the host parasite specificity. He has, on these grounds, reviewed the Habronematinae and regards it as consisting of the following genera : I. *Chitwoodspirura* Chabaud et Rousselot, 1956 ; II. *Sicarius* Li, 1934; III. *Gendrespirura* Chabaud, 1958 ; IV. *Excisa* Gendre, 1928 ; V. *Cyrnea* Seurat, 1914 ; VI. *Habronema* (Diesing, 1861) emended Chabaud, 1958 ; VII. *Odontospirura* Wehr, 1933 ; VIII. *Hadjelia* Seurat, 1916 ; IX. *Histiocephalus* Diesing, 1851 ; X. *Parabronema* Baylis, 1921 and XI. *Draschia* Chitwood and Wehr, 1934.

The Genera of Habronematinae

Chitwood and Wehr established Habronematinae in 1932 and included in it the genera *Habronema, Seurocyrnea, Odontospirura, Parabronema, Hadjelia* and *Draschia*. Chabaud increased the number of the genera of the subfamily from six to eleven. Of the other five, *Sicarius*, an undoubtedly valid genus, was not included in Chitwood and Wehr's paper, perhaps due to its late publication. *Excisa* which was suppressed by Chitwood and Wehr as *Hadjelia*, has rightly been revived by Chabaud as it differs so markedly from *Hadjelia* in its cephalic structure and other characters. *Chitwoodspirura* was erected by Chabaud and Rousselot (1956) for the worm collected from a Gorilla. *Habronema hamospiculum* Baylis, 1931 was transferred to a new genus, *Gendrespirura* by Chabaud in 1958. He also erected a new subgenus *Procyrnea* for all the avian parasites of *Habronema*, which were transferred from the said genus to *Cyrnea*. In this arrangement Chabaud seems to have followed the line proposed by Gendre as far back as 1923. He quotes Gendre who had proposed the separation of the avian and mammalian parasites for convenience sake or "better understanding" although Gendre himself had also accepted the justification of the *Habronema* of birds and mammals being grouped together on the basis of their "sexual characters", in spite of the slight differences in the cephalic ends. Chabaud has also included *Histiocephalus* in Habronematinae. This genus "with wing on each side and with inflation of cervical cuticle to form a collar of longitudinal rib like thickenings, with no papillae of the inner circle and with only four papillae of the outer circle" (Yorke and Maplestone, 1926) can hardly be included in the said sub-family. Yorke and Maplestone (1927), Chitwood and Wehr (1934) and Chitwood and Chitwood (1950) have placed this genus under Schistorophinae, while Skrjabin (1941) has assigned it to Histiocephalinae.

Similarly *Parabronema* was placed under Habronematinae by Chitwood and Wehr (1934) but a glance at its cephalic extremity "with dorsal and ventral cuticular shields, ornamented with six horseshoe shaped cordons", and being the only viviparous genus in the "group", would convince any one that it is a misfit in the subfamily. Even in the key to the genera of the subfamily it is isolated from all others. Yorke and Maplestone had retained it under Acuariinae, while Skrjabin 1941 established a new subfamily Parabronematinae to accommodate it along with *Squamonema*. The combination of the said family with Histiocephalinae and Stellocaronematinae looks very natural.

The Cephalic Extremities of *Habronema*

The cephalic end of *Habronema* has been a matter of controversy for a long time and it was this particular portion which is responsible for the transfer of several worms either from it or to it. The head of this spirurid group has lips which are not only subdivided into lobes, but also have dorsal and ventral accessory projections named by some as lips (Chabaud), by others as interlabia (Chitwood) and by others as cuticular projections (Li).

The differences of opinions about the number of the lips and their accessories can very well be illustrated by quoting the example of *Habronema bulbosum*. Linstow (1906) in reporting *Physaloptera bulbosa*, which was later re-named *Habronema bulbosum*, described it as having six lips. Ortlepp (1925) redescribed the same worm as *Cyrnea bulbosa*, having two lateral lips, each with "a large median lobe and two smaller lateral lobes." Maplestone however declared that the lateral lips are typical and there are "small dorsal and ventral cuticular flaps surrounding the mouth". Baylis (1939) on whose authority the above mentioned accounts are quoted, defines *Habronema* as having "lips entire or trilobed, overlapped by dorsal and ventral cuticular shields." Yorke and Maplestone (1926) gives the definition "mouth with two lips usually trilobed and without teeth, sometimes dorsal and ventral lips in addition." Gendre (1923) also accepts the presence of the two lateral lips and also of a dorsal and a ventral lip. Cram (1927) is however of a different opinion in stating that in *Habronema* the mouth has four lips. Chitwood and Wehr in 1934 defined Habronematinae as having an "oral opening dorsoventrally elongated, surrounded by interlabia and well developed trilobed pseudolabia."

Regarding the arrangement of the cephalic papillae, Chitwood and Wehr were of the opinion that "the four pairs of papillae of the external circle are located at the base of the pseudolabia, the members of each pair (i.e. dorso-dorsals and latero-dorsals) closely approximated to each other" (p.296). As for the papillae of the internal circle, they opined that "each dorso-lateral and ventro-lateral lobe of the pseudolabia bears the rudiment of a papilla of the internal circle". Thus altogether eight papillae of the external and four of the internal circle are said to be present ; in addition to these, there are two amphids. Each one is shown associated with a "papilla like" structure in the *en face* view of *Habronema muscae*.

Osche, in 1955 redescribed *Habronema mansioni* and its cephalic end. His conclusions are as follows : (1) There are two large pseudolips on which are found the latero-dorsal and latero-ventral papillae. (2) On the inner margin of the lips three pairs of teeth are present, and on the outer margin the amphids are located. (3) Two interlabia, one dorsal and the other ventral with median tooth like projections and two papillae dorso-dorsal and ventral-dorsal, are also present.

Chabaud too has described the end-on view of *H. mansioni*. A glance at the two figures of the same structure of the same worm by Osche and Chabaud reminds one of the prophetic saying of Chitwood and Chitwood, that "It is not uncommon for two workers examining the same species to find greater differences than one worker would find examining two representatives of two families." (Introduction to Nematology : Chap. V. Page 56, 1937). The two end-on views though, of one and the same parasite, not only differ in representation, but also in interpretation. The position of the latero-dorsal and latero-ventrals differ in the two figures very markedly. Chabaud's conclusions are as follows :—

(a) "Les quatre grosses papilles latéro-médianes, ainsi que les quatre petites médio-médianes sont toujours sur les lèvres dorsale et ventrale."

He believes that what Li and Osche (p.455-456) regard as dorsal and ventral projections or interlabia, are in fact dorsal and ventral lips, each one with a pair of bigger latero-medians and a pair of smaller medio-medians.

(b) Regarding the labial papillae Chabaud says that,

"La question des papilles du cycle interne est plus difficile à trancher. Il existe effectivement, nettement visibles sur toutes ces espèces, trois points réfringents sur chaque pseudo-lèvre, mais ces points sont plus visibles en profondeur qu'en surface. Nous ne

savons pas s'ils correspondent à l'insertion des trois dents des pseudolèvres, ou si ce sont de véritables papilles sensorielles, qui se termineraient en surface par un filet très mince." (p.465)

Thus Chabaud like Chitwood and Wehr (1934-35) has also observed "the rudiments of papillae of the internal circle, but regards them as insertions of the three pairs of teeth."

The observations of the present writer are almost similar to those of Chitwood and Wehr. The conclusion drawn on the basis of *en face* views of the cephalic ends are as follows : (1) An extra and superficial collar-like structure round the anterior end, with rounded lobes partially overlapping the lateral lips is present. (2) On the dorsal and the ventral planes a triangular projection intrudes between the pseudolabia. (3) There are four pairs of papillae of the external circle ; dorso-dorsals, latero-dorsals, ventro-ventrals and latero-ventrals. (4) There are three pairs of sharp teeth on the internal side of the lips. (5) On each pseudolabium there are three small but fairly conspicuous and sometimes prominent labial papillae. (6) A pair of lateral amphids is present.

A further elaboration of the above is regarded as essential :

(i) The descriptions of the anterior ends by Seurat, Gendre, Maplestone, Walton and others, confirm the presence of a structure that is described by Gendre as cuticle forming a collar round the base of the lips ; by Walton (1923) as a "prepuce partially covering the lips," interpreted by Li (1934), as "a collar-like projection of the cuticle," and described by Maplestone as "a small dorsal and ventral cuticular flap surrounding the mouth." Its presence is also confirmed by Osche (1955) as "cuticular bands." Chabaud however does not make any mention of this structure in his description of *Cyrnea* (*Procyrnea*) *dolichocolpos* Chabaud and Brygoo, 1958. On the basis of the observations on the species to be described, one tends to agree with writers other than Chabaud.

(ii) Chabaud regards the dorsal and the ventral projection of this collar-like structure as the "pédoncule" of the pseudolips. In this view he follows the French savant Gendre who also regards this triangular piece as "accessory lateral development of the lateral lips." The writer's own observations agree with those of Chitwood and Wehr (1934), Chitwood and Chitwood (1937), Li (1935) and Osche (1955) in regarding these structures not only as interlabia, but also as the interlabial projections of the "collar". The shaded areas in the end-on view (see Fig. 2) not only indicate the collar-like

structure surrounding the pseudo-lips but also the dorso-ventral interlabia. Gendre's views that these structures take part in the "occlusion" of the mouth seems to be improbable because they do not seem to extend up to the middle of the mouth cavity. These structures seem to be sensory in function. This interpretation agrees with Chitwood and Wehrs' views of the relationship between various genera of Habronematinae. In *Habronema* the interlabia are least developed and this genus "may reasonably be assumed as the most generalized of the spirurid groups possessing interlabia." In *Draschia megastoma* "the interlabia show a very high degree of development, being in fact taller than the pseudolabia and virtually covering them." In *Cyrnea* this structure is more specialized than in *Draschia*, and finally *Sicarius* seems to be the most specialized of any of them.

(iii) Chabaud regards the dorso-lateral and dorso-ventral papillae as being on the dorsal and the ventral lips, whereas Chitwood and Wehr regard them as located at the base of the pseudolabia. Li reports them to be submedian in position, while Osche regards them as latero-dorsal, but at the base of the pseudolips. The present writer's observation is the same as that of Chitwood and Wehr. As regards the pseudolips my observation varies slightly from that of Osche, and is in agreement with Chitwood and Wehr and also with Li. There seem to be two large lateral pseudolips, each divided into two parts approximately in the middle by the cuticular band, running dorso-ventrally through the lateral plane, with triangular projections—the interlabia—pushed in between the pseudolabia from the dorsal and ventral sides. What Osche has represented as the space between the pseudolips and the bases of interlabia, seems to be the rim of this cuticular band.

(iv) There are divergent views regarding the number and also the position of the cephalic papillae. According to Chitwood and Wehr, there are altogether four pairs of papillae on each lip, a pair consisting of a dorso-dorsal and a latero-dorsal, another pair of ventro-ventral and ventro-lateral, a pair of amphids and a papilla, and the fourth pair with each of its papillae located at the dorso-lateral and the ventro-lateral lobe of the pseudolabium. The last pair is regarded as rudimentary.

Osche (1955) locates the dorso-dorsals and the ventro-ventrals just adjacent to the latero-dorsals and the latero-ventrals. In the end-on view of the same worm, *H. mansioni*, Chabaud's representation of these papillae is different from that of Osche, the two papillae

being quite separate. In the specimens under study the approximation of the dorso-dorsal and their counterparts on the ventral side seem to be in confirmation of Chitwood and Wehr and also of Osche's observations. Li does not seem to have located the small dorso-dorsal and dorso-ventrals. In the presence of the three pairs of papillae of the inner circle, the present observations are in complete agreement with those of Li and also with Chitwood and Wehr. What the later writers have located near the amphid in the end-on view of *H. muscae* seems to be the interno-lateral papilla itself. There seem to be variations in its approximation with the amphid. In some species it is between the two papillae of the inner circle, while in others, it is located in the mid lateral region of the pseudolip, while in still others it is found very close to the amphid. Chabaud's view that these papillae are insertions of teeth does not seem to be convincing, particularly in view of the displaceable position of the interno-laterals. Osche's pressure experiment might have obliterated the small labial papillae from view.

Habronema and *Cyrnea*

Chitwood and Wehr (1934) allotted all the mammalian parasites of this group to *Habronema* and the avian worms to *Cyrnea* (*Seurocyrnea*). Gendre, as described by Chabaud, had also suggested the separation of these two "groups" of parasites according to their hosts. Chabaud seems to have followed the same scheme and separated the avian parasites from those of mammalia, but he himself does not seem to have been convinced of this separation. That is why in spite of the fact that all the species of *Cyrnea* are parasites of birds, the avian species of *Habronema* could not be merged with them as species of one and the same genus and he had to maintain two different subgenera ; i.e. one, *Cyrnea* for the original species and the other, *Procyrnea* for accommodation of the avian species of *Habronema*. In the writer's opinion this arrangement seems to be too artificial, for the following reasons. Host parasite specificity, though important, should not be regarded as a decisive factor in the classification of a parasite unless it is supported by morphological evidence. It is quite true that several worms specialize in parasitising particular hosts, but there are many examples of worms living on more than one group of animals as their hosts. The following examples are quoted from Chitwood and Wehr (1934):

Parasite	Host	Location
Gongylonema	Ma. Av.	Es. C. Mo.
Hedruris	R. Am.	S.
Cosmocephalus	Pi. Av.	G.
Spiroxys	Am. R.	S.
Hartertia	Av. Ma.	I. G.
Physaloptera	Ma. R. Av.	S. I.
Abbreviata	R. Ma.	Mo. S. I.
Thubunaea	R. Ma.	I. S.
Proleptus	Pi. ?R.	I. S.
Cucullanus	Pi. R.	I.
Camallanus	Pi. Am.R.	S. I.

Ma. mammals ; Av. birds ; R. reptiles ; Am. amphibians ; Pi. pisces ; Mo. mouth ; I. intestine ; S. Stomach ; G. gizzard ; Es. oesophagus ; C. crop.

All the genera mentioned above belong to Spiruroidea alone. More examples can also be quoted from other super-families. The adaptibility of worms to new hosts and new habitats provides them with better chances of survival. Hence it is not advisable to differentiate between parasites of the same genus on the basis of host parasite specificity alone. Morphological characters, rather than the host, would be a better and more sound criterion for such a classification. If the host parasite specificity causes morphological changes in the parasite, it should certainly be regarded as a basis for splitting the group into subgenera, as has been done in *Physaloptera*. In this case, all the subgenera are retained in the same genus. If it is considered advisable to divide *Habronema* into two groups, these should be retained as subgenera of *Habronema* and not transferred to *Cyrnea*.

There seems to be little justification for the partitioning of *Habronema*, because no major morphological difference exists between the *Habronema* parasites of birds and mammals. A comparative study of the characters of *Habronema*, *Procynea* and *Cyrnea*, as described by Chabaud indicates more differences between *Procyrnea* and *Cyrnea*, than between *Procyrnea* and *Habronema*.

"In having cervical papillae far posterior to the nerve ring ; with vulva post equatorial ; with ovejector having a vestibule ; with parallel uteri ; with symmetrically arranged postanal papillae ; without lateral flange and without the group of sessile papillae near the tip of the tail ;" *Cyrnea* has been established with least doubt, as a valid genus ; it is of course very closely related to *Habronema*.

The inclusion of the avian *Habronema* under this genus having different characters from *Cyrnea*, would not be justified. Even in the structure of the cephalic end the interlabia of the two genera differ from each other.

The only character which could have been a valid basis for partitioning *Habronema* is the reported displacement of the vulva in some of the mammalian *Habronema*, towards the dorsal side. In all the specimens of *H. muscae* and *H. clarki* that were examined, in the London School of Hygiene and Tropical Medicine, the body was either twisted or distorted, hence the exact position of the vulva could not be determined ; however, the acceptance of its ventral position in *H. microstoma* makes the earlier statements rather doubtful. Even if the displacement of the vulva is to be regarded as a fact, the justication would be to keep both the mammalian and the avian parasites under *Habronema* alone as its subgenera. Hence for convenience as suggested by Gendre, a new subgenus *Aviabronema* is proposed for the reception of the avian parasites of this genus.

Habronematinae Chitwood and Wehr, 1932

I. **Habronema** Diesing, 1861

Generic diagnosis : Mouth with dorsoventral opening surrounded by interlabia and well developed trilobed pseudolabia; a collar-like projection of cuticle around the head end; papillae of external circle, 8 in number, dorso-dorsals and latero-dorsal, ventro-ventrals and latero-ventrals closely approximating one another ; papillae of inner circle, 6 in number, reduced in size ; cervical papillae anterior to nerve ring ; body without or with lateral alae. Male with unequal spicules ; postanal papillae usually asymmetrical ; group of sessile papillae near the tip of tail; gubernaculum present. Females with vulva in median region of the body. Oviparous; parasites of mammals and birds.

Key to subgenera of *Habronema*

Vulva on the ventral axis or displaced dorsally. Only one lateral ala present. Parasites of mammals ..... *Habronema* subgen. nov.

Vulva on ventral axis, body without or with lateral alae. Parasites of birds..... *Aviabronema* subgen. nov.

Of the species described so far, *H. circi* Li, 1934 is regarded by Chabaud as a synonym of *H. leptopterum*, but the characters of the

two species differ considerably. Hence it is regarded as a valid species. *H. indicum, H. euplocami, H. imbricatum, H. diesingi* and *H. casuarii* described by Maplestone, in fact belong to the genus *Cyrnea*. *H. khalili*, reported by Ezzat Abd Elmoneim (1945) has rightly been placed under *Excisa* by Chabaud (1958). A number of spirurids have been reported as parasites of *Rhea americana* by several writers, under various generic names ; Freitas and Lent (1947) have synonymised them all as *Habronema uncinipenis* Molin, 1860. A new species has also been reported as *Sicarius waltoni* which has been renamed as *C.* (*P.*) *waltoni* by Chabaud (1958). The only difference between *H. uncinipenis* Molin, 1860 and *C.* (*P.*) *waltoni* (Freitas and Lent, 1947) is in the ratio of the spicules, which is 4 : 1 in the former, and 8 : 1 in the latter. Some of the species reported by Johnston and Mawson (1941), *C. paraleptoptera* and others, could not be included in the key as their description is too vague.

Habronema (*Aviabronema* subgen. nov.) *mansioni* Seurat, 1914

Five specimens of this species, three males and two females, were obtained once from the proventriculus of the pale harrier, *Circus macrourus*. The worms were found underneath the horny layer. As the host is a new record, a description of the specimen is given here.

These are medium sized worms with a body, in both sexes, of uniform diameter, attenuated only near the anterior and the posterior ends, and more so towards the latter. There is a slight difference in size of the two sexes, the males being thinner.

The cuticle is finely striated transversely. Two lateral alae are seen in the anterior region of the body of the male. The alae are unequal, the left being 3 mm. long and the right 2·5 mm. In the female, however, the alae are feebly developed, measuring 1·8 mm. in length. The cervical papillae are not very far ahead of the nerve ring, the two being at a distance of 0·2–0·21 mm. and 0·24–0·26 mm. from the head end in the male and 0·22–0·24 mm. and 0·26–0·28 mm. in the female. The excretory pore opens at 0·28 mm. and 0·38 mm. from the anterior tip of the body in the two sexes respectively.

The two lateral lips surrounding the mouth are fairly broad, each lip with trilobed margin possesses three fine teeth and three minute papillae. One pair, each consisting of a well developed and a smaller papilla is seen near the dorsal and the ventral margin of each pseudolabium. In between these pairs a laterally situated amphid is

also observed. The collar-like projection at the base of the lips, partially overlap the pseudolips, with their submedian lobes. The dorsal and the ventral tongue-like projections protrude into the mouth cavity in between the pseudolabia. The mouth opens into a short thick walled pharynx, measuring 25μ in length and 15μ in breadth in the male, and 28μ by 18μ in the female. The anterior and the posterior portions of the oesophagus, measure 0·56–0·78 mm. and 2·45–2·56 mm. in length, respectively in the male and 0·62–0·68 mm. and 3·12–3·61 mm. in the female. The entire oesophagus, 3·0–3·34 mm. long in the male and 3·74–3·29 mm. in the female, occupies almost one-third of the total body length.

Male. The males are 9·8–10·2 mm. long and have a maximum width of 0·20–0·23 mm. The tail is 0·22–0·26 mm. long. A pair of wide, asymmetrical caudal alae surround the tail, the left being three times as long as the right. The two measure 0·83–7 mm. and 0·63–0·71 mm. in length and 0·5–0·08 mm. and 0·03–0·05 mm. in breadth. There are four pairs of pedunculated, symmetrically arranged, precloacal papillae, with an isolated papilla situated immediately in front of the cloaca on its left side. There are four more pedunculated postcloacal papillae, of which the two posterior ones are symmetrical in arrangment and of the first two, the one on the left is lateral in position while the other one is midventral. In addition to these there are four more pairs of sessile papillae near the tip of the tail, of these the second and the third pairs form two groups of two each, the first and the last one marginals. The spicules are very unequal, the left being nearly three times longer than the right, and measuring 0·70–0·98 mm. and 0·32–0·39 mm. respectively. An accessory piece, with a maximum breadth of 52μ by 48μ is also observed. The tip of the left spicule is pointed, that of the right is blunt and knob-like.

Female. The females are 12–15·7 mm. long with a maximum breadth of 0·32–0·36 mm. The vulva which is somewhat protruded from the body wall opens into a sac-like muscular vagina, 0·44–0·49 mm. long. The vulva is equatorial, at a distance of 6·96–7·12 mm. from the tip of the tail. The eggs are thick shelled, embryonated and measure 35μ by 21μ. The tail is 0·124 mm. long terminating in a knob-like swelling.

Host : *Circus macrourus*

Habitat : Proventriculus

Locality : Hyderabad, India

Habronema (*Aviabronema*) *ameerae* n.sp.

On two occasions when the Indian kestrel, *Cerchneis tinnunculus interstinctus* was searched for parasites, a pair of *Habronema* was collected. They were recovered from the gizzard in which they were found lying under the horny layer.

These are comparatively small worms, but the difference in the size of the two sexes is well marked. The body is yellowish-white when alive, the cuticle is transversely striated. Lateral alae could not be observed even after prolonged search and are regarded as absent. The cervical papillae are seen ahead of the nerve ring at a distance of 0·13–0·14 mm. from the head end in the male and 0·154–0·16 mm. in the female. Both are located at the same level. The excretory pore opens at the level of the nerve ring which encircles the oesophagus at 0·17–19 mm. from the anterior tip in the male and 0·19-0·21 mm. in the female.

The head bears two small lateral lips, each somewhat trilobed. Each lip possesses three labial papillae and an equal number of pointed teeth. The cephalic papillae are eight in number, quite prominent and are encircled by the submedian lobes of the collar-like projection, so characteristically present in the genus. A pair of amphids is situated laterally. The tongue-shaped triangular chitin-like plates are dorso-ventral in position. The pharynx is 0·025 mm. long and 0·017 mm. wide in the male and approximately the same size in the female. The oesophagus measures 3·03–3·28 mm. in length in the male, and 3·3–3·6 mm. in the female, and constitutes slightly less than half the length of the body, the glandular region of the oesophagus being unusually long. The two component portions measure 0·42–0·47 mm. and 2·61–2·81 mm. in the male, and 0.49–0·51 mm. and 2·91–3·12 mm. in the female.

Male. The male is 4·6–5·3 mm. long, with a maximum thickness of 0·19–0·21 mm. The tail has symmetrical caudal alae 0·51–0·55 mm. long by 0·31–0·40 mm. wide. The cloaca is 0·18–0·21 mm. from the tip of the tail. There are four pairs of precloacal, and two pairs of postcloacal caudal papillae, all pedunculated and symmetrical. In addition there are four pairs of sessile papillae near the tip of the tail. The spicules are unequal ; the left is 0·78–0·89 mm. in length and the right 0·28–0·31 mm. The right spicule has its tip curved inwardly like a hook. The gubernaculum is small, having a maximum length and breadth of 25–30μ by 23–29μ.

Female. The female is 8–8·9 mm. long, with a maximum thickness of 0·23–0·29 mm. The vulva is post-equatorial, being 3·21–

3·62 mm. from the tail end. The vagina is flush with the body, has a thick muscular wall and is 0·32–0·39 mm. in length. The uterus is tightly packed with thick shelled embryonated eggs measuring 31μ by 21μ. The tail is 0·14 mm. long.

Relationships. The present worm being devoid of lateral alae resembles *H.* (*A.*) *ficheuri* Seurat, 1916, *H.* (*A.*) *murrayi* Ortlepp, 1934 and *H.* (*A.*) *aegotheles* Johnston and Mawson, 1941. The first two differ from it being longer, in possessing sessile papillae in front of the cloaca, in having longer spicules with spear-shaped ends and with different arrangement of the caudal papillae. *H.* (*A.*) *aegotheles* is the smallest of the three and is half as long as the worm under discussion. While the left spicule of the two are approximately of the same length, the right spicule of *H.* (*A.*) *aegotheles* is three times longer than that of the present worm. The arrangement of the preanal papillae is also different in the two. While there are four and six preanal papillae irregularly arranged in the former, there are four papillae on each side of the latter. The length of the female tail and that of the eggs also vary in the two.

H. (*A.*) *leptopterum* Seurat, 1914 and *H.* (*A.*) *spinosum* Gendre, 1922 were reported from the same host in Europe and Africa. The former seems to possess lateral alae on both sides and the latter species has a single lateral ala. In both species the alae are described as prominent. *H.* (*A.*) *spinosum* has approximately the same length as that of the present worm, while *H.* (*A.*) *leptopterum* is more than one and a half times longer. It differs from *H.* (*A.*) *spinosum* in not having spines, in having a post equatorial vulva, longer spicules, different types of spicule tips, in not possessing precloacal sessile papillae and in body measurements. In view of these differences, it is regarded as a new species and it is proposed to name it *Habronema* (*Aviabronema*) *ameerae* after my student Miss Ameer Sultana.

Host : *Cerchneis tinnunculus interstinctus*
Habitat : Proventriculus
Locality : Andhra Pradesh, India.

Habronema (*Aviabronema*) *suraiyae* n.sp.

Six male specimens of this worm were collected from the proventriculus of a red-headed merlin, *Falco chicquera*. The worms were found lying under the horny layer.

These are fairly stout worms 11·9–12·3 mm. long and 0·38–0·42 mm. maximum breadth in the middle of the body. The head is

not marked off from the body and has a maximum diameter of 0·07–0·08 mm. The posterior end is sharply attenuated. The cuticle has well marked transverse striations. The lateral ala is present on the left side only, starting at the level of the nerve ring, and extending to the middle of the body. The nerve ring and the excretory pore are at a distance of 0·29–0·31 mm. and 0·49–0·46 mm. respectively from the anterior end. The cervical papillae are bristle-like, situated at 0·22–0·26 mm. from the head end in front of the nerve ring. The head is somewhat rounded, and the lips are trilobed. Each of the two large lateral lips bears three minute papillae, two prominent dorso-dorsal and ventro-ventral and two smaller latero-dorsal and latero-ventral papillae, and an amphid. A cuticular collar-like projection is found at the base of the lips. There are three minute teeth on each lateral lip.

The pharynx is cylindrical and measures about 0·023 mm. long and 0·011 mm. wide. The muscular and the glandular portions of the oesophagus are 0·71–0·76 mm. and 2.61–2.83 mm. long, and occupy about one-quarter of the total length of the body.

The caudal end of the parasite is curved ventrally. The caudal alae are comparatively short, but wide, being 0·42 mm. in length and 0·25 mm. in width. There are four pairs of preanal, lateral papillae and a pair of sessile papillae, ventro-lateral in position, situated immediately in front of the cloacal aperture. Two pairs of post-cloacal, pedunculated papillae and three pairs of small sessile papillae, are also observed near the tip of the tail. The spicules are unequal, but not thickly chitinized. The left spicule is thin and long, with expanded head and a pointed tip. The right spicule is comparatively thicker, but has a similar head and tip. They measure 0·93–1·12 mm. and 0·37–0·41 mm. long respectively. A gubernaculum is present, and measures 49μ in length and 23μ in width. The tail is 0·22–0·26 mm. long.

Relationships. The present material resembles *H.* (*A.*) *tulostoma* (Hemprich and Ehrenberg, 1866) Gendre, 1922, *H.* (*A.*) *urophasiana* Wehr, 1933 and *H.* (*A.*) *uncinipenis* in possessing a single lateral ala and in having a length of closer range. It differs from the last one in having a larger lateral ala, in the spicule ratio, and in the body length which is half as long as the latter. *H.* (*A.*) *urophasiana* is longer than the present worm but has a shorter lateral ala, and with four times longer spicules. *H.* (*A.*) *tulostoma* has the same spicule ratio and also a lateral ala of the same size, but it differs from the present worm in several respects. *H.* (*A.*) *tulostoma* was described by Schneider, 1866 ; Seurat, 1922 and Gendre, 1922. In Table I the comparative measurements and features indicate that the worm under discussion is not

the same as *H. tulostoma* as described by these authors. Hence it is regarded as a new species.

Table I

	Schneider, 1866 mm.	Seurat, 1922 mm.	Gendre, 1922 mm.	Present Material *H.* (*A.*) *suraiyae* n.sp. mm.
Length	17	8	13·9–16·7	11·2–11·8
Spicules		1·9 0·6	1·7 –1·8 0·51–0·55	0·93–1·12 0·37–0·41
Tail		0·3		0·2
Caudal alae		1·23 long ·300 wide		·42 long 0·25 wide
Precloacal sessile papillae		One		One pair
Postcloacals		Two pairs of sessile papillae, ventral in position.		Two pairs of pedunculated papillae lateral in position
Papillae at the tip of the tail		Six		Eight

Seurat regarded his specimen to be *H.* (*A.*) *unilateralis* but Gendre was of the opinion that the two species are separate. Cram agrees with Gendre and the opinion of the present writer is the same. The material under study differs from *H.* (*A.*) *unilateralis* in the lengths of body, cervical ala, spicules and caudal alae. The presence of a single sessile precloacal papilla is another distinguishing character. In view of the above mentioned facts, the worm collected from the red-headed merlin is regarded as new, and named as *Habronema* (*Aviabronema*) *suraiyae* after my student Suraiya Alvi.

Host : *Falco chicquera*

Habitat : Proventriculus

Locality : Hyderabad, India

Habronema (*Aviabronema*) *singhi* n.sp.

Several males and females of this worm were collected from the proventriculus of the common pariah kite, *Milvus migrans govinda*.

The male measures 10·2–12·9 mm. in length and 0·34–0·36 mm. in maximum thickness, the female 13·3–14 mm. and 0·36–0·38 mm.

respectively. The cuticle is transversely striated, having sharply projecting edges. The posterior end of the body is more sharply attenuated than the anterior. The mouth is surrounded by fairly large rounded lateral trilobed pseudolips. Each lip bears three minute teeth at its edge. There are present three small papillae near the margin of each lip and at the dorsal and ventral margin of their bases, there is a pair of dorso-dorsal and latero-dorsal and ventro-ventral and latero-ventral papillae of which the latter two in each group are feebly developed. The amphid is seen laterally. Surrounding the lips at their bases runs a collar-like projection, giving off four submedian lobes and two dorso-ventral lobes. The submedian lobes harbouring the papillae are tongue-shaped, while the dorso-ventral ones are triangular and project towards the mouth inbetween the pseudolabia. The mouth opens into a thick walled pharynx measuring 31μ long and 12μ broad. The nerve ring encircles the oesophagus at a distance of 0·20–0·22 mm. from the anterior tip in the male and 0·26–0·26 mm. in the female. The excretory pore is seen at 0·32–0·34 mm. in the male and 0·36–0·38 mm. in the female. The cervical papillae are anterior to the nerve ring being at a distance of 0·14–0·16 mm. from the head end in the male and 0·17–0·19 mm. in the female. There is a very narrow and short lateral ala on the left side of the body in both sexes. The oesophagus is 2·6–3·9 mm. long in the male and 4·2–4·7 mm. in the female. The component portions, in the two sexes measure 0·79–0·81 mm. and 2·82–3·12 mm., and 0·62–0·81 mm. and 3·63–3·91 mm. respectively. The oesophagus occupies one-quarter of the entire body length in the male and one-third in the female.

Male. The males are more slender than the females and their ventrally flexed tails possess well developed symmetrical alae measuring 0·62–0·81 mm. long and 0·04–0·06 mm. broad. The cloaca is situated at 0·2–0·31 mm. from the tip. There are four pairs of pedunculated precloacal papillae with a fairly big sessile papilla immediately in front of the cloacal aperture on its left side. The post-cloacal pedunculated papillae are two pairs in number, of which the first is asymmetrical, the right being mid-ventrally situated. Near the tip of the tail there are altogether six pairs of sessile papillae of which the first is comparatively bigger than the rest; the second, third and the fourth form two groups of three pairs each. The fifth and sixth pairs of sessile papillae are immediately anterior and posterior to the pair of phasmids. The tip of the tail projects out of the caudal alae. The spicules are unequal and dissimilar, the left one is 0·99–1·3 mm. long and the other 0·36–0·48 mm. The gubernaculum

measures 27μ long and 17μ wide. The smaller spicule has an inwardly curved tip.

Female. The dimensions of the female are not very markedly different from those of the male. The tail is 196μ long and ends in somewhat rounded tip. The vagina opens just behind the middle of the body, being at a distance of 5·9–6·1 mm. from the posterior end. The muscular vagina is 0·46–0·49 mm. long and the uterus is tightly packed with thick shelled, embryonated eggs, measuring 25μ long by 15μ wide.

Relationships. H. (*A.*) *leptopterum* Seurat, 1914 and *H.* (*A.*) *mansioni* Seurat, 1914 are the two species reported from kites (*Milvus*). The present worm collected from the common pariah kite in India differs from both the species in several characters as indicated in Table II.

Table II

All measurements are in millimetres except eggs

	H. (*A.*) *leptoptera*	*H.* (*A.*) *mansioni*	*H.* (*A.*) *singhi* n.sp.
Body length ♂	7–10	9–11	9·8–10·2
♀	14	13–17	12·1–13·7
Lateral alae	Two; one fourth the width of the body	Two wide; in anterior half of the body	One; narrow; very short
Spicules	0·64 and 0·2	0·68 and 0·31	0·99–1·3 and 0·36–0·48
Caudal papillae	4 pairs preanal. No sessile preanal. 2 pairs asymmetrical postanal 8 sessile at tip of tail.	4 pairs preanal. One sessile preanal. 2 pairs postanal symmetrical. 8 sessile at tip of tail.	4 preanal. One sessile preanal. 2 pairs postanal asymmetrical. 12 sessile at tip of tail.
Caudal alae	Wide, short and symmetrical.	Asymmetrical	Narrow, long and symmetrical.
Eggs	32μ × 20μ	28 μ× 19μ	25μ × 15μ

Thus it could not be assigned to either of these species. It resembles *H.* (*A.*) *uncinipenis* in having one lateral ala in the position of the vulva, but *H.* (*A.*) *uncinipenis* is twice as long as the present worm. The spicule ratio and their lengths vary very much in the two. Hence it is regarded as a new species and named after Professor S. N. Singh as *Habronema* (*Aviabronema*) *singhi*.

Host: *Milvus migrans govinda*
Habitat: Proventriculus
Locality: Hyderabad, India

Habronema (*Aviabronema*) *chabaudi* n.sp.

The following account of a new species of *Habronema* is based on material consisting of a single pair, collected from the proventriculus of the Indian falcon, *Falco jugger*.

These are medium sized worms, with little difference in the size of the two sexes. The male and female measure 11·9 mm. and 15·9 mm. respectively in length and 0·40 mm. and 0·42 mm. in maximum thickness. The cuticle is markedly striated transversely. In the anterior region where the striations are more prominent than those of the posterior region, their projections have a fine needle-like appearance. The cervical papillae are much in front of the nerve ring and are situated at the same level, at a distance of 0·17 mm. from the head end in the male and 0·19 mm. in the female. The excretory pore opens in the vicinity of the nerve ring which is at a distance of 0·41–0·46 mm. from head end in the male and female respectively. The lateral ala is narrow and short. Starting posterior to the cervical papillae on the right side, it runs backwards for 2·18 mm.

The head possesses two lateral lips, each one provided with three teeth and three small labial papillae, and four pairs of the papillae of the external circle. The cuticular collar-like structure round the head is quite prominent and also the laterally situated phasmids. The pharynx has well chitinized walls, 26μ long by 15μ wide. The oesophagus occupies one-quarter of the entire body length, measuring 3·4 mm. in the male and 3·8 mm. in the female. Its component portions are 0·62 mm. and 2·82 mm. and 0·78 mm. and 3·13 mm. long in the two sexes respectively.

Male. The males have well developed broad caudal alae, covered with cuticular shields in longitudinal series, as are found in all the species of *Habronema*. The caudal alae are symmetrical and are 0·65 mm. long and 0·51 mm. broad. There are four pairs of precloacal and two pairs of postcloacal pedunculated papillae; these are crooked like the head of a golf club. There is a pair of sessile papillae immediately in front of and a similar pair just posterior to the cloacal aperture. In addition to these there are four pairs of small sessile papillae near the tip of the tail, of which the first and third are ventro-lateral and the second and fourth are lateral in position. The spicules are unequal in size but similar in shape; both have expanded heads, tubular bodies and sharply pointed tips. The right spicule is 0·40–0·46 mm. long whereas the left measures 1·67–1·81 mm. in length. The gorgeret is small, 23μ by 15μ.

Relationships. In possessing two pairs of sessile papillae, one anterior and one posterior to the cloaca, this worm differs from all the known species of *Habronema*. It differs from *H.* (*A.*) *leptopterum* a parasite reported from the genus *Falco*, in several characters. *H.* (*A.*) *leptopterum* possesses two lateral alae and asymmetrical cervical papillae ; it is smaller in length (males 7·1 mm., females 14 mm.) ; it does not possess sessile papillae on either side of the cloaca ; it has symmetrical postanal papillae ; it has smaller spicules (0.64 mm. and 0·2 mm. as against 1·47–1·68 mm. and 0·4–0·46 mm.) and has a pre-equatorial vulva. In all the above mentioned characters the present worm differs from it. From *H.* (*A.*) *monopterum* which has spicules of almost similar length it differs in body length, in the position of the vulva, in the arrangement of the caudal papillae, in having symmetrical cervical papillae and a short narrow lateral ala. *H.* (*A.*) *urophasiana* of somewhat similar length differs in having spicules four times longer than those of the worm under discussion. Not only the spicule ratio, but the number of pre- and post-cloacal sessile papillae and the arrangement of the other caudal papillae, differ in the two. Hence it is regarded as a new species. It is proposed to name it *H.* (*A.*) *chabaudi* after Professor Alain G. Chabaud.

Host : *Falco jugger*

Habitat : Proventriculus

Locality : Hyderabad, India.

Habronema (*Aviabronema*) *monoptera* Gendre, 1922

Three males of this species were once recovered from the proventriculus of the tawny eagle, *Aquila vindhiana*. These are thin, medium sized worms measuring 7·8–8·1 mm. in length and 0·21–0·25 mm. in maximum thickness. The cuticle has coarse transverse striations having sharply projecting edges giving the body an imbricated appearance. The lateral ala is present on the left side in the anterior region, starting at the level of the anterior end of muscular portion of the oesophagus and running backward almost up to one third of the body. The cervical papillae are in front of the nerve ring, at a distance of 0·136 mm. from the head end. The excretory pore opens at the level of the nerve ring at 0·16–19 mm. from the head end. The mouth is surrounded by well developed rounded lateral lips each possessing three pairs of teeth and three small labial papillae. The outer ring of papillae consists of four pairs of papillae and a laterally

situated amphid. The characteristic cuticular collar-like ring surrounding the bases of the lips is present, with four quadrangular projections on its lateral position and a triangular process on each dorsal and ventral side.

The thick walled pharynx is almost as wide as long being 25μ by 23μ wide. The muscular and the glandular portions of the oesophagus are 0·39–0·41 mm. and 2.31–2·63 mm. long respectively. The entire oesophagus which is 2·7–3 mm. long occupies one-third of the total body length.

The tail bears asymmetrical caudal alae, of which the right is 0·92 mm. long and the left 0·77 mm. long. The tip of the tail protrudes beyond the caudal alae. The cloacal aperture is 0·22–0·26 mm. from the posterior end of the body. There are four pairs of preanal caudal papillae. Both the preanal and the postanal papillae are feebly developed and while the preanal papillae on the right are equidistant the left preanals are arranged in two groups, the first and second papillae forming the first group and the third and fourth forming the second. There are two pedunculated postanal papillae on the left side, but there is only one on the right. A solitary midventral pedunculated papillae can be seen between the first and second postanals. Near the tip of the tail there are five pairs of sessile papillae ; the first pair consists of one papilla on each side, but the subsequent four pairs are arranged in two groups of two pairs each.

The spicules are similar in shape but unequal in size, the left being 1·86–1·90 mm. and the right 0·38–0·44 mm. long ; they are tubular and have sharply pointed tips. The accessory piece has a length and breadth of 35μ and 32μ respectively.

Host : *Aquila vindhiana*

Habitat : Proventriculus

Locality : Hyderabad, India

Habronema (*Aviabronema*) *daleri* n.sp.

Two male and three female worms of this species were collected from the gizzard of the green pigeon, *Crocopus phoenicopterus chlorogaster*. They were found attached to the walls of the gizzard under the horny layer. The two sexes do not exhibit marked differences in length or breadth, the male measuring 8·0–8·9 mm. by 0·19–0·21 mm. and the female, 10·2–11·1 mm. by 0·21–0·29 mm. in length and breadth respectively. The body is comparatively thin and the

colour of the worm when collected, is yellowish-white. The cuticle is striated transversely. The mouth bears two lateral lips, each provided with three teeth near its anterior margin. There are three pairs of small papillae near the inner margin of the lip. Forming the base of the lips, is a collar-like structure with four submedial lobes, on each of which is found a pair of papillae, one big and one small. In the *en face* view the dorso-ventral interlabia appear as keel-like projections. A little distance behind the head end a fairly broad cervical ala is present on one side of the body, running backwards a little distance beyond the terminus of the glandular portion of the oesophagus and occupying one-third of the body length. The buccal capsule has fairly thick chitinized walls and is as wide as long, measuring 0·028 by 0·026 mm. in the male and 0·031 by 0·028 mm. in the female. The cervical papillae are located at 0·13–0·14 mm. from the tip of the head in the male and 0·19–0·29 mm. in the female. The excretory pore opens at the level of the nerve ring, at a distance of 0·21–0·26 mm. and 0·29–0·33 mm. from the anterior tip in the male and the female respectively. The oesophagus measuring 2·87–3·51 mm. and 3·33–3·61 mm. in length in the two respective sexes forms one-third of the total body length, in both male and female. The muscular and the glandular portions of the oesophagus in the two sexes are 0·36–0·39 mm. and 2·51–3·12 mm. and 0·41–0·49 mm. and 2·92–3·12 mm. long respectively.

Male. The tail is 0·20–0·25 mm. long and ends in a blunt point. A pair of well developed slightly asymmetrical caudal alae is present, measuring 0·62 long on one side and 0·74 on the other, the maximum span being 0·07–0·09 mm. The ventral side of the tail has characteristic longitudinal ridges. There are five pairs of pedunculated caudal papillae, of which four pairs are preanal and one pair postanal and asymmetrical. The precloacal papillae on the left side are somewhat asymmetrically arranged, attached to the body on the ventro-lateral plane, the posterior two are more markedly so. Between the cloaca and the postanal papillae, there is a group of three well developed sessile papillae. Another group of sessile but smaller papillae is seen just anterior to the tip of the tail ; these are eight in number, arranged in three pairs and one pair, the fourth being just near the tip. The first pair is situated almost at the mid-region between the pedunculated postanal papillae and the tip of the tail, and between the first and fourth pairs, the second and third pairs of sessile papillae are seen, one of these being sub-ventral and the other lateral in position. The spicules are very unequal, measuring 0·99–1·21 mm. and 0·32–0·41 mm. long. The gubernaculum is present and is 0·033 mm. long by 0·022 mm. wide.

Female. The females are slightly longer than the males. The vulva is situated anterior to the middle of the body, being at a distance of 7·2–7·3 mm. from the tip of the tail, which is 0·1 mm. long. The muscular vagina measures 0·49–0·51 mm. in length. The eggs are embryonated, measuring 30μ in length and 21μ in width. The tail is 0·18–0·02 mm. long.

Relationships. In possessing a pre-equatorial vulva and somewhat similar length, the present worm comes close to *H.* (*A.*) *magnilabiatum* Maplestone, 1932, *H.* (*A.*) *monopterum* and *H.* (*A.*) *asymmetricum* Maplestone, 1932, but it differs from them in several respects. The position of the vulva in *H.* (*A.*) *monopterum* is stated to be equatorial as well as pre- and post-equatorial. The present worm differs from it in having smaller spicules with greater ratio, in not having a hook-like process near the tip of the spicule, in not having precloacal sessile papillae, in having a group of three postcloacal sessile papillae, in possessing a muscular vagina half as long, with a lateral ala twice the length of the latter, and the female tail of double the length. *H.* (*A.*) *magnilabiatum* has a much shorter and narrower lateral ala and has all the post-caudal papillae symmetrically arranged and lacks the group of sessile papillae at the tip. The lips of *H.* (*A.*) *magnilabiata* are broad and those of the present species very narrow. The teeth are reported to be absent in the first and are present in the latter. In *H.* (*A.*) *asymmetricum* the caudal alae are very asymmetrical, the caudal papillae quite irregularly placed, and some are even double. The vulva in the present worm is much anterior to the middle of the body. In *H.* (*A.*) *unilateralis* which also resembles the present worm, the spicule ratio and the position of the vulva differs ; moreover the left spicule is alate in the former and non-alate in the latter and the arrangement of the papillae in the two alae differ. Hence it is regarded as a new species and named *H.* (*A.*) *daleri* after my colleague Dr. Daler Khan, at the London School of Hygiene and Tropical Medicine.

Host : *Crocopus phoenicopterus chlorogaster*

Habitat : Gizzard

Locality : Hyderabad, India.

Habronema (*Aviabronema*) *hyderabadensis* n.sp.

Numerous worms of this species were collected from the grackle, *Gracula intermedia.* The worms were found under the horny layer of the gizzard and also in the proventriculus. This is a comparatively thin worm with tapering posterior ends in both sexes.

There is little difference in the length of the two sexes, the males being differentiated by their thinner body and the ventrally curved tail. The males are 10·3–11·1 mm. long 0·18–0·22 mm. wide. The bodies in both sexes are transversely striated and possess lateral alae on both sides in the anterior region. The wide alae start right from the base of the lips and in both sexes run as far back as the posterior third of the body.

The mouth is surrounded by two large lateral lips each possessing three small teeth and three fairly well developed papillae. The papillae of the outer circle, two pairs on each lip are even more prominent, somewhat posteriorly placed, and adjacent to them rise the lateral alae. Enclosing the papillae in their tongue-shaped submedian folds, is the collar-like projection so characteristically present in the species under observation. The triangular chitin-like interlabia are seen wedged in between the lips, on the dorsal and ventral side. A pair of lateral amphids can be seen in the end-on view.

The cephalic papillae are far anterior, almost midway between the head and the nerve ring, being at a distance of 0·13–0·15 mm. from the anterior tip in the male and 0·14–0·16 mm. in the female. The nerve ring and the excretory pore are found at 0·27–0·31 mm. and 0·29–0·33 mm. and 0·16–0·36 mm. and 0·37–0·38 mm. from the head end, in the male and female respectively.

The mouth opens into a well chitinized buccal capsule, 0·028–0·030 mm. long and 0·015 mm. wide in the male and 0·030–0·031 mm. long by 0·017 mm. wide in the female. Both the base and the apex of the pharynx are thicker than the walls. The oesophagus, in both sexes, occupies one-quarter of the entire body length, being 2·97–3·5 mm. long in the male and 3·38–3·63 mm. in the female. The muscular and the glandular portions are 0·36–0·56 mm. and 2·61–2·91 mm. and 0·49–0·51 mm. and 2·89–3·12 mm. in both sexes.

Male. The tail is spirally coiled and possesses wide, well developed caudal alae, 1·18 mm. long and 0·19 mm. wide. The spicules are very unequal. The left measures 1·2–1·6 mm. in length and the right 0·34–0·41 mm.; both of them end in a bluntly pointed tip. The gubernaculum is 35μ by 15μ. The cloacal aperture is 0·36–0·41 mm. from the tip of the tail which is rounded, nodule shaped and extends beyond the caudal alae. There are four pairs of preanal papillae with a pair of sessile papillae situated just in front of the cloaca. The postcloacal papillae are of two types, pedunculated and sessile. Immediately behind the cloaca there is a pair of asymmetrical papillae of which the left one is lateral and the right mid-ventral in position. The second pair of the postcloacals is to be seen

at the end of the first one-third of the tail. There are four pairs of small sessile papillae near the posterior tip of the body. Of these the first and the fourth pair are somewhat isolated from the other two which form a group, in which one pair is ventro-lateral and the other is lateral in position. A pair of phasmids is observed between the last and second group of sessile papillae.

Female. The female has an almost equatorial vulva, opening at a distance of 6·1–6·4 mm. from the tip of the tail. The muscular vagina is 0·28–0·31 mm. long. Both the branches of the uterus and the vagina are fully packed with thick shelled embryonated eggs measuring 32μ long by 16μ wide. The tail is rather long, measuring 0·35 mm.

Relationships. In having a pre-equatorial vulva, a rather long left spicule and a similar body length, the present worm resembles *H.* (*A.*) *colaptes*, but it differs from it in the length and ratio of the two spicules, in having a wide and very long lateral ala, in possessing a pair of preanal sessile papillae, symmetrical caudal alae, in the longer tail in the female and in the elliptical eggs. *H.* (*A.*) *mansioni*, in which the position of the vulva is stated to be precloacal, also has smaller spicules and symmetrical postcloacal papillae, with asymmetrical caudal alae shorter than that of the present worm. It is regarded that a new species is being dealt with and it is proposed to name it *H.* (A.) *hyderabadensis*.

Host : *Gracula intermedia*

Habitat : Gizzard

Locality : Hyderabad, India.

Habronema (*Aviabronema*) *waheedae* n.sp.

Out of the five specimens of *Habronema* collected from the proventriculus of the brahmany kite, *Haliastur indus*, there were three males and two females. The only differentiating character between the two sexes is the alate tail in the male ; the size of the body is almost similar in the two sexes.

The worms are of medium size with a range of 9·1–9·9 mm. in length, and 0·19–0·21 mm. in breadth in the male and 10·9–11·2 mm. and 0·21–0·23 mm. respectively in the female. The cuticle has

coarse striations with sharply projecting edges, giving the body an imbricated appearance. The lateral alae are present on both sides of the body, the left being more developed than the right which is very narrow and fringe-like. The left ala starts from the base of the cervical papillae (0·15–0·16 mm. from the head end in the male and 0·16–0·17 mm. in the female), and runs backwards up to 3·2 mm. in the male and 4·2 mm. in the female. The nerve ring and the excretory pore are located at a distance of 0·23–0·26 mm. and 0·16–0·18 mm and 0·25–0·27 mm. and 0·27 and 0·29 mm. from the head end in the male and the female respectively.

The mouth is surrounded by two small pseudo-labia, trilobed, each bearing three teeth on their internal surface. Three papillae of the internal circle and two pairs of papillae of the external circle with a laterally situated amphid, are also seen on each lip. The collar-like projection, with submedian and tongue-like triangular dorso-ventral interlabia is also to be observed in the *en face* view. The pharynx has a thick chitinous wall and is 21μ long and 14μ wide. The muscular and the glandular portions of the oesophagus measure 0·41–0·44 mm. and 2·6–2·8 mm. in length in the male and 0·46–0·49 mm. and 2·91–3·12 mm. in the female. The entire oesophagus, 3·0–3·2 long in the male and 3·3–3·61 mm. in the female, occupies one-third of the body length.

Male : The tail is 0·22–0·25 mm. long and carries well developed symmetrical caudal alae 0·52–0·56 mm. long and 0·1–0·2 mm. broad. The spicules are unequal, the right being one-third as long as the left, the two measuring 0·32–0·36 mm. and 0·91–0·98 mm. in length. The left spicule ends in a pointed tip ; the right is stouter and has an inwardly directed hook-like tip. The small gubernaculum is 27μ by 13μ in maximum length and breadth. The caudal papillae consist of six pedunculated and four sessile pairs. Of the former, four pairs are precloacal and two are postcloacal. While the first pair of the postcloacals is located near the cloacal aperture, the second postcloacal is near the group of sessile papillae. The last mentioned are found in three groups of one pair, two pairs and one pair each. The caudal alae do not enclose the tip of the tail which protrudes and ends in a pointed tip.

Female. The vulva is flush with the body and opens into a "7"-shaped muscular vagina, 0·35–0·38 mm. long. Both the uterus and the vagina are tightly packed with embryonated eggs 29μ and 27μ broad. The vulva is 5·8–6·1 mm. from the tail end and is almost equatorial. The tail is sharply pointed, and is 0·19 mm. long.

Relationships. In possessing thin, longish, pedunculated postanal papillae, widely set apart, this material differs from all the known species of *Habronema*. In the absence of the precloacal sessile papilla, symmetrical postcloacals and pre-equatorial vulva, it resembles *H.* (*A.*) *americanum* and *H.* (*A.*) *magnilabiatum*. The latter species which is also reported from India, differs from the present worm in having a single lateral ala, much longer spicules, and the female is almost one and a half times longer. Moreover the lips of *H.* (*A.*) *magnilabiatum* are much wider and it possesses a broad lateral ala. The arrangement of the postanals differs very much in the two. *H.* (*A.*) *americanum* has smaller spicules, asymmetrical caudal alae and narrow and shorter cervical alae. The lips of the present worm are narrow and that of *H.* (*A.*) *americanum* broad. In view of the facts mentioned above, the worm collected from the Indian brahmany kite is regarded as a new species. It is proposed to name it *H.* (*A.*) *waheedae* after my wife.

Host : *Haliastur indus*

Habitat : Proventriculus

Locality : Hyderabad, India.

Habronema (*Aviabronema*) *magnipapillatum* n.sp.

Two male and three female worms of this species were collected from the proventriculus of the butastur, *Butastur teesa*. Of these five specimens, only one male and one female were entire ; of the rest either the posterior or the anterior halves were available for study The parasites were found embedded underneath the horny layer of the proventriculus.

These are rather large and thick worms, the males being 10·2–12·2 mm. long and 0·38–0·42 mm. thick, and the females 12·9–13·8 mm. and 0·44–0·49 mm. in length and breadth respectively. The extremities are not markedly attenuated ; the posterior, which is sharper than the anterior, ends in a somewhat swollen tip. The cuticle possesses transverse striations, with backwardly projecting edges. There are two lateral alae one on each side of the anterior portion of the body. The right ala is 3·6–3·8 mm. long in the male and 4·8–5·2 mm. in the female and the left is 2·6–3·8 mm. in the male and 4·0–4·2 mm. in the female. The cervical papillae which are thick and stumpy, are seen almost midway between the anterior end and the nerve ring, at a distance of 0·18–0·20 mm. in the male and 0·19–0·21 mm. in the female. The excretory pore opens adjacent to the

nerve ring which encircles the anterior portion of the oesophagus at about 0·42–0·46 mm. from the head end in the male and 0·46–0·49 mm. in the female.

The mouth is surrounded by two large pseudolabia, each being somewhat trilobed and possessing three pairs of teeth, three small labial papillae and two pairs of the papillae of the outer circle. The cuticular collar forming the base of the lips, harbours the dorso-dorsals and the ventro-ventrals, each accompanied by a smaller papilla. The amphid is lateral; the dorso-ventral triangular inter-labia protrude towards the mouth cavity. The thick walled pharynx is quite prominent, measuring 42μ in length and 26μ in breadth. The oesophagus is one-quarter the body length in the male and one-third in the female, being 2·67–3·37 mm. and 4·2–4·7 mm. long in the two respective sexes. Its constituent portions are 0·61–0·56 mm. and 2·61–2·81 mm. and 0·71–0·81 mm. and 3·71–3·91 mm. long in the male and female respectively.

Male. There is little difference between the two sexes in size. The male is however recognised by the ventrally curved tail, 0·23–2·34 mm. long and surrounded by well developed asymmetrical caudal alae. The right caudal ala is longer than the left, the two measuring 0·71–0·73 mm. and 0·63–0·68 mm. long respectively. The maximum expanse of the two alae ranges from 0·39–0·52 mm. There are six pairs of pedunculated caudal alae of which four are preanal in position. Of the sessile papillae at the tip of the tail, there are six pairs, of which the third and fifth are laterally disposed, the second and sixth are almost mid-ventral and the fourth pair is ventro-lateral. The spicules are dissimilar in shape. The left is tubular, and measures 0·93–1·24 mm. in length. The right is 0·34–0·46 mm. long and possesses an inwardly curved tip. The gubernaculum is small with a length and breadth of 0·064 mm. and 0·043 mm. respectively. The pedunculated papillae are prominent and thickest of all the species reported in this communication.

Female. The females are slightly stouter than the males. The vulva is pre-equatorial in position being 6·88–7·41 mm. from the tip of the tail. The muscular vagina which is 0·49–0·52 mm. long, possesses lips protruding from the body. The uterus and its two limbs are tightly packed with embryonated eggs, measuring 36μ by 22μ the tail is 300μ long and ends in a knob.

Relationships. The present worm is closely allied to *H.* (*A.*) *waheedae* n.sp. reported in this paper; but in having robust caudal

papillae, a prominent and protruding vulva, six pairs of sessile papillae at the tip of the male tail and a much longer tail in the female it differs from the latter. The eggs of the former are oval and bigger whilst those of the latter are smaller and elliptical. The two also differ in the arrangement of the papillae. *H.* (*A.*) *magnilabiatum* which resembles the present worm in not possessing the precloacal papilla, differs from it in the length and ratio of the spicules, in possessing a single cervical ala and in various measurements. *H.* (*A.*) *americana* having no precloacal sessile papillae, but with symmetrical caudal alae, smaller spicules and the position of the vulva much anterior to the middle of the body and with a single pair of post-cloacal papillae, is differentiated from the species under study. Hence it regarded as new and named *H.* (*A.*) *magnipapillatum* n.sp.

Host : *Butastur teesa*

Habitat : Proventriculus

Locality : Hyderabad, India.

Key to Species of *Habronema* Diesing, 1861

Habronema subgen. nov. mihi.

1. Spicules more than 1 mm.; vulva "displaced dorsally";..... 2
   Spicules shorter ; vulva on or near the ventral axis..... 3

2. Vulva about the ant. third of body ; cervical papillae at the level of nerve ring ; spicule ratio 5 : 1 (L.S. 2·5 mm. R.S. 0·5 mm.).....*H.* (*H.*) *muscae* (Carter, 1861)

   Vulva in front of the ant. third of body ; cervical papillae in front of the nerve ring ; spicule ratio 4 : 1 (L.S. 1·7–1·9 mm., R.S. 0·4–0·56 mm.)... *H.* (*H.*) *zebrae* Theiler, 1924

3. Vulva on ventral axis ; cervical papillae at the level of nerve ring ; male tail without sessile subterminal papillae .....*H.* (*H.*) *microstoma* (Schneider, 1866)

   Vulva slightly displaced from ventral axis ; cervical papillae anterior to nerve ring ; male tail with sessile subterminal papillae.....*H.* (*H.*) *clarki* Foster and Chitwood, 1937

Aviabronema subgen. nov. mihi

1. Body without lateral alae 2
   Body with one or two lateral alae 5

2. Males more than 6 mm.; females more than 10 mm.; subterminal papillae present 3
   Males less than 6 mm.; females less than 10 mm.; subterminal papillae absent 4

3. Caudal alae long and symmetrical; preanal pap. 4 pairs; spicule ratio 4 : 1 (L.S. 1.26 mm. R.S. 0·35 mm.) L.S., with two barbs. ..... *H.* (*A.*) *ficheuri* Seurat, 1916
   Caudal alae broad and asymmetrical; preanal pap. 4–6; spicule ratio 3 : 1 (L.S. 0·88–0·89 mm. R.S. 0·32–0·33 mm.), L.S. with one barb only ..... *H.* (*A.*) *murrayi* Ortlepp, 1934

4. Caudal alae symmetrical; males 4·5–5·3 mm., females 5·3 mm.; spicule ratio 3 : 1 (L.S. 0·78–0·89 mm. R.S. 0·28–0·31 mm.); preanal pap. 4 pairs; eggs 31μ×21μ ..... *H.* (*A.*) *ameerae* n.sp.
   Caudal alae asymmetrical; males 2·5 mm. females 8·1–8·9 mm.; spicule ratio 7 : 1 (L.S. 0·78 mm. R.S. 0·1 mm.); preanal pap. 4–6; eggs 45μ×20μ ..... *H.* (*A.*) *aegotheles* Johnston and Mawson, 1941

5. One lateral ala present 6
   Two lateral alae present 19

6. Males more than 10 mm. long 7
   Males less than 10 mm. long 12

7. Lateral ala long 8
   Lateral ala short 10

8. Lateral ala one third body length; spicules long (L.S. 4·1 mm. R.S. 1·1 mm.) ..... *H. A.*) *urophasiana* Wehr, 1931
   Lateral ala half the body length; spicules short 9

9. Males 17 mm.; females 19·22 mm.; L.S. 1·9 mm.; R.S. 0·6 mm.; caudal alae very long; single sessile papilla in front of cloaca ..... *H.* (*A.*) *tulostoma* (Hemprich and Ehrenberg, 1866)
   Males 11·2 mm.; females unknown; L.S. 0·9–1·1 mm; R.S. 0·37–0·41 mm.; caudal alae short and wide; a pair of sessile papillae in front of cloaca ..... *H.* (*A.*) *suraiyae* n.sp.

10. Vulva at the posterior quarter of body ; spicule ratio 7 : 1 (L.S. 2·4–2·6 mm.; R.S. 0·3–0·4 mm.) .....*H.* (*A.*) *uncinipenis* Walton, 1928
Vulva post equatorial..... 11

11. Lateral ala very narrow ; spicule ratio 3 : 1 (L.S. 0·99–1·3 mm.; R.S. 0·36–0·48 mm.) cloacal pap. absent ; post cloacal papillae asymmetrical.....*H.* (*A.*) *singhi* n.sp.
Lateral ala $^1/_6$th of the body length ; spicule ratio 4 : 1 (L.S. 1·6–1·8 mm.; R.S. 0·4–0·46 mm.) two pairs of sessile cloacal papillae ; post anal papillae symmetrical ..... *H.* (*A.*) *chabaudi* n.sp.

12. Lateral ala broad..... 13
Lateral ala narrow..... 16

13. Caudal papillae irregularly placed ; some papillae double .....*H.* (*A.*) *asymmetricum* Maplestone, 1932
Preanal papillae regularly placed ; postanals asymmetrical 14

14. Precloacal sessile papillae absent ; three postanal mid-ventral papillae present ; spicule ratio 3 : 1 (L.S. 0·99–1·2 mm.; R.S. 0·32–0·41 mm.) lateral ala one third body length.....*H.* (*A.*) *daleri* n.sp.
Precloacal sessile papillae present..... 15

15. Spicule ratio 2·5 : 1 ; L.S. alate ; lateral ala moderate ..... *H.* (*A.*) *unilateralis* (Molin, 1860)
Spicule ratio 4 : 1 (L.S. 1·5–1·6 mm.; R.S. 0·38–0·46 mm.) both spicules nonalate ; lateral ala $^1/_3$rd body length ..... *H.* (*A.*) *monopterum* Gendre, 1922

16. Vulva pre-equatorial ; spicule ratio 3 : 1 (L.S. 1·78 mm.; R.S. 0·55 mm.)..... *H.* (*A.*) *magnilabiatum* Maplestone, 1932
Vulva post-equatorial ; spicule ratio 4 : 1..... 17

17. Cuticle with spines ; L.S. 1·2–1·3 mm.; R.S. 0·34–0·4 mm.; L.S. with spur.....*H.* (*A.*) *spinosum* Gendre, 1922
Cuticle without spines, left spicule acicular..... 18

18. Preanal sessile papilla present ; spicules alate ; postanal papilla asymmetrical..... *H.* (*A.*) *pileata* Walton, 1928
Preanal sessile papilla absent ; spicules nonalate ; postanal papillae symmetrical (1 pair sessile) .....*H.* (*A.*) *imbricatum* Maplestone, 1932

19. Males 10 mm. or longer ; vulva pre-equatorial..... 20
Males smaller ; vulva pre- or post- equatorial..... 27

20. Males 13 mm.; females 18 mm. long ; L.S. very long (L.S. 6·3 ; R.S. 0·6 mm.)
..... *H.* (*A.*) *dolichocolpos* (Chabaud and Brygoo, 1958)
Males and females smaller ; spicules smaller..... 21

21. Vulva behind the first third of the body ; lateral alae unequal, one about 1 mm., the other 5 mm.
..... *H.* (*A.*) *incertum* (Smith, 1908)
Vulva pre-equatorial..... 22

22. Left spicule more than 1 mm. long..... 23
Left spicule less than 1 mm. long..... 24

23. Spicule ratio 5 : 1 (L.S. 2 mm.; R.S. 0·4 mm.) lateral alae narrow ; single precloacal sessile papilla present
.....*H.* (*A.*) *colaptes* Walton, 1923
Spicule ratio 4 : 1 (L.S. 1·2–1·6 mm.; R.S. 0·34–0·41 mm.) lateral alae wide ; a pair of precloacal sessile papillae present..... *H.* (*A.*) *hyderabadensis* n.sp.

24. Precloacal sessile papillae present ; postcloacal papillae asymmetrical..... *H.* (*A.*) *mansioni* Seurat, 1914
Precloacal sessile papillae absent ; postcloacal papillae symmetrical 25

25. Lateral alae end anterior to nerve ring ; spicules smaller ; lips broad.....*H.* (*A.*) *americanum* Chandler, 1941
Lateral alae and spicules longer ; lips small..... 26

26. Vulva prominent ; caudal papillae robust ; 6 pairs of subterminal papillae ; female tail long (0·3 mm.)
..... *H.* (*A.*) *magnipapillatum* n.sp.
Vulva not prominent ; caudal papillae feeble, 4 pairs of subterminal papillae ; female tail small (0·19 mm.)
.....*H.* (*A.*) *waheedae* n.sp.

27. Body rolled in spiral ; wide lateral alae
..... *H.* (*A.*) *longistriata* (Molin, 1859)
Body straight ; lateral alae narrow..... 28

28. Left spicule more than 2·5 mm. long..... 29
Left spicule much smaller..... 30

29. Left spicule 3·24 mm ; right spicule 1·08 mm ; spicule ratio 3 : 1.....*H.* (*A.*) *LONGISPICULUM* Walton, 1928

Left spicule 2·75 mm.; right spicule 0·35 mm.; spicule ratio 8 : 1..... *H.* (*A.*) *SKRJABINI* Tschernikowa, 1934

30. Vulva equatorial ; lateral ala ½ of the body length ; spicule ratio 2 : 1 (L.S. 0·55–0·58 mm ; R.S. 0·24–0·25 mm.) .....*H.* (*A.*) *CIRCI* Li, 1934

Vulva pre-equatorial ; lateral alae ¼ of the body length..... 31

Vulva post equatorial ; lateral alae feebly developed..... 32

31. Lateral alae equal ; spicule ratio 3 : 1 (L.S. 0·64 mm.; R.S. 0·2 mm.).....*H.* (*A.*) *LEPTOPTERUM* (Rud., 1819)

Lateral alae unequal ; spicule ratio 1·5 : 1 (L.S. 0·36–0·41 mm.; R.S. 0·2–0·28 mm.)....*H.* (*A.*) *BUCKLEYI* Bisseru, 1955

32. Vulva at ⅔ body length ; spicule ratio 5 : 1 (L.S. 2·1 mm.; R.S. 0·45 mm.)..... *H.* (*A.*) *SEURATI* Skrjabin, 1917

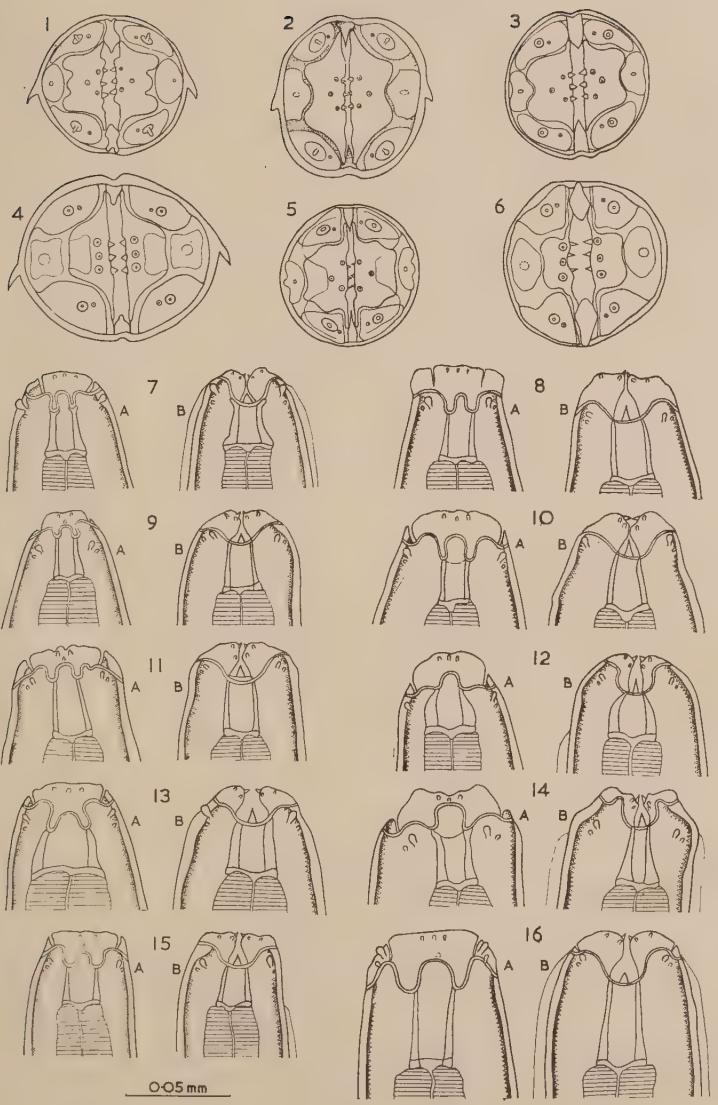
Vulva near posterior end of the body ; spicule ratio 7 : 1 (L.S. 1·7 mm.; R.S. 0·29 mm.)..... .....*H.* (*A.*) *DIESINGI* Maplestone, 1932

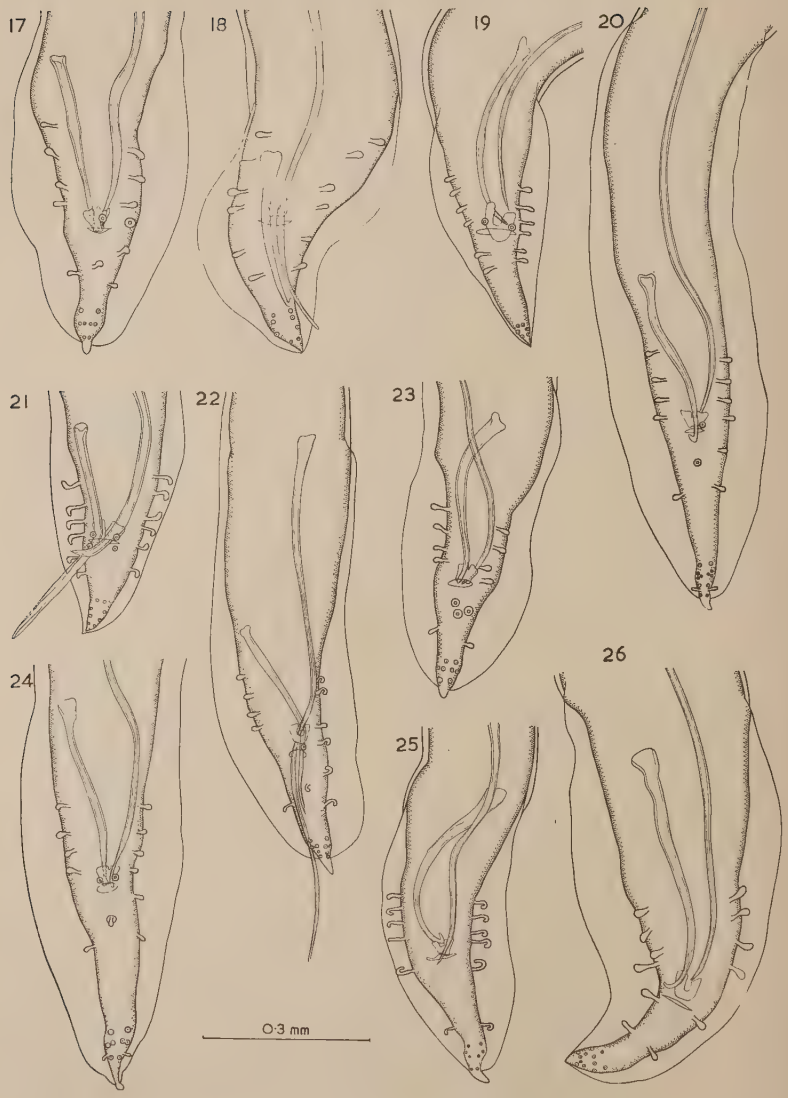
End-on Views

Fig. 1.—*Habronema* (*Aviabronema*) *mansioni*. Fig. 2.—*H.* (*A.*) *chabaudi* n.sp. Fig. 3.—*H.* (*A.*) *daleri* n.sp. Fig. 4.—*H.* (*A.*) *hyderabadensis* n.sp. Fig. 5.—*H.* (*A.*) *waheedae* n.sp. Fig. 6.—*H.* (*A.*) *magnipapillatum* n.sp.

Cephalic extremities (*a*) *lateral view*, (*b*) *dorsal view.*

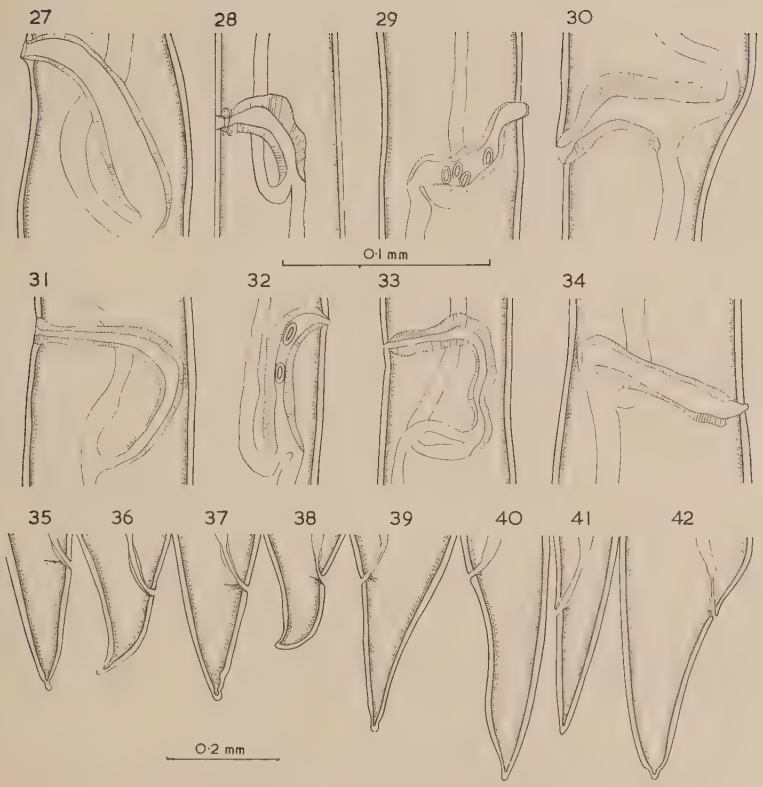
Fig. 7.—*H.* (*A.*) *mansioni* Seurat, 1914. Fig. 8.—*H.* (*A.*) *ameerae* n.sp. Fig. 9.—*H.* (*A.*) *suraiya* n.sp. Fig. 10.—*H.* (*A.*) *singhi* n.sp. Fig. 11.—*H.* (*A.*) *chabaudi* n.sp. Fig. 12.—*H.* (*A.*) *monopterum* Gendre, 1922. Fig. 13.—*H.* (*A.*) *daleri* n.sp. Fig. 14.—*H.* (*A.*) *hyderabadensis* n.sp. Fig. 15.—*H.* (*A.*) *waheedae* n.sp. Fig. 16.—*H.* (*A.*) *magnipapillatum* n.sp.


1
2
3
4
5
6
7
A
B
8
9
10
11
12
13
14
15
16
0·05 mm



Male tails, ventral view

Fig. 17.—*H.* (*A.*) *mansioni*. Fig. 18.—*H.* (*A.*) *ameerae*. Fig. 19.—*H.* (*A.*) *suraiyae*. Fig. 20.—*H.* (*A.*) *singhi*. Fig. 21.—*H.* (*A.*) *chabaudi*. Fig. 22.—*H.* (*A.*) *monopterum*. Fig. 23.—*H.* (*A.*) *daleri*. Fig. 24.—*H.* (*A.*) *hyderabadensis*. Fig. 25.—*H.* (*A.*) *waheedae*. Fig. 26.—*H.* (*A.*) *magnipapillatum*.



Vulva, vagina and uterus of female worms.

Fig. 27.—*H.* (*A.*) *mansioni*. Fig. 28.—*H.* (*A.*) *ameerae*. Fig. 29.—*H.* (*A.*) *singhi*. Fig. 30.—*H.* (*A.*) *chabaudi*. Fig. 31.—*H.* (*A.*) *daleri*. Fig. 32.—*H.* (*A.*) *hyderabadensis*. Fig. 33.—*H.* (*A.*) *waheedae*. Fig. 34.—*H.* (*A.*) *magnipapillatum*.

Lateral view of tails (female).

Fig. 35.—*H.* (*A.*) *mansioni*. Fig. 36.—*H.* (*A.*) *ameerae*. Fig. 37.—*H.* (*A.*) *singhi*. Fig. 38.—*H.* (*A.*) *chabaudi*. Fig. 39.—*H.* (*A.*) *daleri*. Fig. 40.—*H.* (*A.*) *hyderabadensis*. Fig. 41.—*H.* (*A.*) *waheedae*. Fig. 42.—*H.* (*A.*) *magnipapillatum*.

II **Excisa** Gendre, 1928

General diagnosis. Pseudolips well developed, each divided into three conical lobes, interlocking with those of the opposite side. Dorsal and ventral interlabia notched ; cephalic papillae, 8 of the outer circle, and 6 of the inner circle ; cervical papillae posterior to nerve ring ; lateral ala absent ; stoma without cuticular collar ; postanal papillae symmetrical ; spicules unequal ; gubernaculum present ; vulva equatorial. Parasites of birds, mammals (?) and reptiles (?).

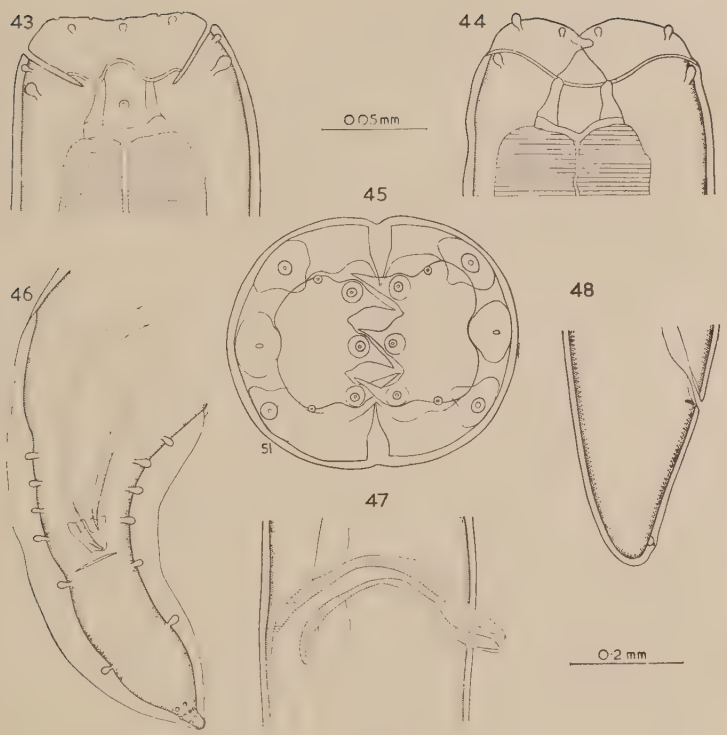
Excisa buckleyi n.sp.

The white necked stork, *Dissoura episcopus*, was once dissected for worms and was found to be infested by a new species of *Excisa*. The parasites were collected from the gizzard and the proventriculus, under the horny layer.

These are large, thick worms with a maximum width of 0·35–0·38 mm. in the male and 0·38–0·42 mm. in the female. The body tapers only at the extremities, more so at the posterior. The head has a maximum diameter of 0·09–0·10 mm. and 0·11–0·12 mm. respectively in the male and female. The cuticle has coarse transverse striations with sharp edges, which begin at the base of lips. The cervical papillae are 0·27 mm. (left) and 0·29 mm. (right) in the male and 0·28 mm. and 0·32 mm. in the female, from the anterior end. The nerve ring and the excretory pore are located at 0·19–0·21 mm. and 0·26–0·28 mm. from the anterior end in the male, 0·21–0·23 mm. and 0·29–0·31 mm. in the female.

The mouth is surrounded by two large speudolips deeply notched and divided into three triangular tooth like projections interlocking with their counterparts. At the base of each triangular process, there is a very prominent papilla. The dorsal and ventral interlabia are short and also notched. There are four pairs of papillae of the outer circle. The pharynx has a well chitinized wall but is very short, measuring 42μ in maximum breadth and 22μ in total length. The oesophagus occupies $\frac{1}{4}$ of the body length in both sexes, being 4·6–4·8 mm. long in the male and 5·6–5·9 mm. in the female. The muscular and glandular portions are 0·39–0·41 mm. and 4·36–4·39 mm. and 0·41–0·48 mm. and 5·18–5·46 mm. respectively in the male and female.

Male. The males are 17–18·6 mm. long. The tail is 0·36–0·41 mm. long and has a pair of broad and well developed symmetrical alae, 0·92–1·21 mm. long 0·36–0·39 mm. broad. The ventral surface



Excisa buckleyi n.sp.

Fig. 43.—Anterior end, lateral view. Fig. 44.—Anterior end, dorsal view. Fig. 45.—End-on view. Fig. 46.—Posterior end, male, ventral view. Fig. 47—Equatorial region, female, lateral view. Fig. 48.—Posterior end, female, lateral view.

of the tail is marked by longitudinal ridges interrupted by irregular transverse bands. There are four pairs of preanal and two pairs of postanal papillae. The preanal papillae are all symmetrical. The first postanal lies a little behind the cloaca, while the second pair is almost equidistant between the cloaca and the tip of the tail. There are four pairs of smaller pedunculated papillae symmetrically arranged near the tip. The spicules are similar in shape but very unequal in length, the left being four times as long as the right, and measure 1·69–1·87 mm. and 0·48–0·51 mm. in length. Both have broad heads and pointed tips. The gubernaculum is 0·066 mm. long while its minimum and maximum breadth is 0·026 mm. and 0·072 mm. respectively.

Female. The females measure 19·9–23·9 mm. in length. The vulva protrudes from the body wall and has two prominent lips. It is equatorial in position being at a distance of 10.8–12 mm. from the tip of the tail. The vagina is muscular, 0·59–0·63 mm. long, and the two ovaries are situated in the post-oesophageal region and the last quarter of the body respectively. The uteri are packed with thick shelled, embryonated eggs measuring 43μ by 24μ. The tail which ends in a broad rounded tip is 0·26 mm. long. Just anterior to the tip of the tail there is a pair of papillae with spike like projections.

Relationships. This genus was revived by Chabaud in 1958 and at present includes three species, i.e. *E. excisa* (Molin, 1860) ; *E. dentifera* (Johnston and Mawson, 1940), *E. khalili* (Ezzat Abd Elmoniem, 1945). *E. khalili* was found in a bottle with parasites of rhinoceros, but Chabaud expresses doubt that the rhinoceros can be the host. He is of the opinion that *Ciconia* must be the host and that the material was misplaced. Linstow had reported *Excisa excisa* from a snake *Tropidonotus hydrus*, but Seurat had expressed doubt about this host also. Taking into consideration the fact that *Habronema* can parasitize both mammals and birds, it is desirable at present, not to express any opinion till more is known about the distribution of this genus.

The present worm differs from others of this genus in the length and ratio of the spicules, the arrangement of papillae, the absence of precloacal sessile papillae, the presence of the four pairs of papillae near the tip, in having a prominent vulva, and the longest tail in the female which bears a pair of papillae near the tip ; hence it is regarded as a new species and named after Professor J. J. C. Buckley, as *Excisa buckleyi* n.sp.

	E. excisa	*E. dentifera*	*E. khalili*	*S. buckleyi* n.sp.
Length ♂	16	10	16	17·9–18·6
♀	19–26	16	20	19·8–23·9
Spicules L.	2·6	2·7	1·09	1·69–·192
R.	·62	0·5	0·39	0·45–0·51
Papillae	4 pairs preanal	4 pairs preanal	4 pairs preanal	4 pairs preanal
	1 unpaired sessile preanal	—	—	—
	2 postanal	2 postanal, 1 immediately postcloacal and the other ½ way between cloaca and tip	2 postanal	2 postanal, 4 pairs at the tip of the tail
♀ tail	0·3	0·22	0·27	0·37–0·41

All measurements in mm.

Host : *Dissoura episcopus*

Habitat : Proventriculus and gizzard

Locality : Hyderabad, India.

III **Cyrnea** Seurat, 1914

Generic diagnosis. Habronematinae : stoma without a mediodorsal and a medioventral cuticular projection ; interlabia well developed, projecting as phalangiform processes between pseudolabia ; cephalic papillae, 4 pairs of outer circle, and three pairs of inner circle ; lateral ala absent ; cervical papillae posterior to nerve ring ; spicules unequal ; gubernaculum present ; postanal papillae symmetrical ; subterminal papillae pedunculate and symmetrical ; vulva post-equatorial ; oviejector with vestibule ; parasites of birds.

Cyrnea singhi n.sp.

Four males of this species were once collected from the proventriculus and gizzard of *Centropus sinensis.*

These are small worms with a rather thick body, measuring 5·4–6·1 mm. in length and 0·32–0·4 mm. in thickness. The cuticle is not markedly striated and the lateral alae are absent. The head bears two pseudolips and a pair of well developed interlabia. The pseudolabia possess four pairs of papillae of which two pairs are dorsodorsal and latero-dorsal and the other two ventro-ventral and latero-ventral in position. Three pairs of papillae comprise the inner circle, and lateral amphids are present. Only two pairs of teeth could be detected. The nerve ring surrounds the muscular portion of the oesophagus at a distance of 0·18–0·21 mm. from the head end. The excretory pore opens at the level of the cervical papillae at 0·25–0·29 mm. from the anterior tip.

The mouth leads into a short and feebly chitinized vestibule measuring 70μ in length and 19μ in width. The oesophagus is divided into two parts, the anterior being 0·17–0·19 mm. long and the posterior 1·42–1·64 mm. The entire oesophagus, 1·59–1·82 mm. in length, occupies $^1/_3$ of the body length.

The tail is surrounded by well developed symmetrical caudal alae measuring 0·32–0·34 mm. long and 0·25–0·27 mm. wide. There are altogether nine pairs of caudal papillae ; of these, three pairs of preanal in position. The first pair of postanal papillae is immediately

behind the cloaca and the second pair is isolated and somewhat anterior to the middle of the tail. Near the tip of the tail there are four pairs of small but pedunculated papillae symmetrically arranged. The spicules are similar in shape and measure 1·16–1·21 mm. and 0·71–0·74 mm. in length. A small gorgeret 46μ by 24μ wide is also present. Both the preanal and the postanal papillae on the right, are attached to the ala rather than to the body. The cloaca opens at 0·14 mm. from the tip of the tail.

Relationships. The present worm is the smallest of the species so far reported in the genus *Cyrnea*. It resembles *C. eurycerca* Seurat, 1914, *C. indica* Maplestone, 1929 and *C. colini* Cram, 1927, so far as body length is concerned, but differs from them as follows :—

1. In having a shorter right but much longer left spicule ; in possessing a much smaller oesophagus ; in the absence of a pair of papillae just in front of the cloaca and in the arrangement of the caudal papillae, the present worm differs from *C. eurycerca*.

2. *C. indica* has much smaller spicules and a different disposition of the caudal papillae and possesses asymmetrical caudal alae. In addition, *C. indica* is longer.

3. *C. colini* has a much longer left spicule, and the right spicule is half as long as that of the present worm. *C. colini* has ten pairs of caudal papillae with quite a different arrangement.

C. semilunaris (author ?) of somewhat similar length, is very vaguely described, as having "unequal spicules, with nine caudal papillae of which six are very long," hence it is not possible to compare the two.

In view of the characters discussed above, this is regarded as a new species. It is proposed to name it *C. singhi* after Professor S. N. Singh of Osmania University, India.

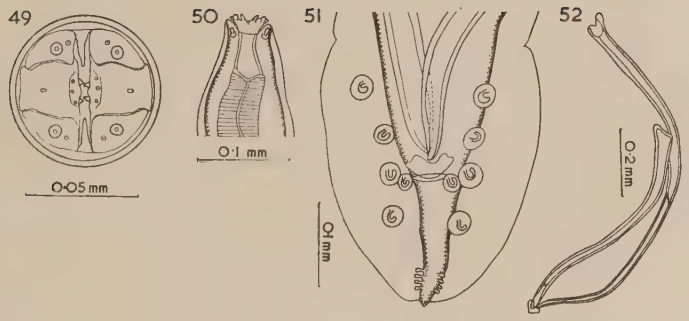
Host : *Centropus sinensis*

Habitat : Proventriculus and gizzard

Locality : Hyderabad, India.

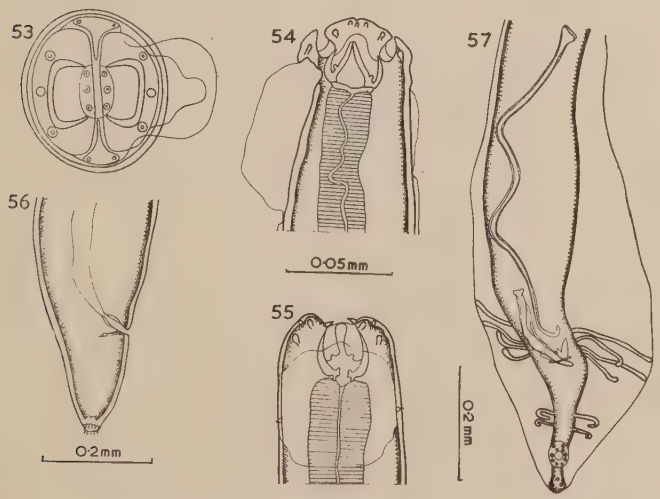
IV. **Sicarius** Li, 1934

Generic diagnosis. Habronematinae. Stoma with pseudolabia and a pair of dorso-ventral lip like structures, all provided with papillae ; mouth cavity with a pair of chitin like plates ; cephalic papillae, 8 of outer circle and 6 of inner circle ; cephalic collar absent ;



Cyrnea singhi n.sp.

Fig. 49.—End-on view. Fig. 50.—Anterior end, lateral view. Fig. 51.—Posterior end, male, ventral view. Fig. 52.—Spicules.



Sicarius dipterum Li, 1939.

Fig. 53.—End-on view. Fig. 54.—Anterior end, lateral view. Fig. 55.—Anterior end, dorsal view. Fig. 56.—Posterior end, female, lateral view. Fig. 57.—Posterior end, male, lateral view.

cervical papillae anterior to nerve ring; caudal papillae long and phalangiform; subterminal sessile papillae arranged in a circle. Vulva equatorial; female tail with a cluster of cuticular processes; parasites of birds.

Sicarius dipterum Li, 1934

This species was originally described by Popowa in 1927 as *Habronema dipterum*. Li in 1934 erected a new genus, *Sicarius*, for which he took *dipterum* as the type species. The present worm collected from the same host, *Upupa epops*, agrees with the type species in general, but possesses certain morphological differences. Hence it was thought advisable to redescribe it.

A large number of both males and females were collected from the small intestine of a hoopoe, *Upupa epops*. The two sexes differ considerably in size, both in length and in maximum width. In both sexes the anterior half of the body is thinner than the posterior. Both the males and females possess lateral alae, which occupy the whole length of the body. In the anterior region, just behind the head, the cuticle is asymmetrically inflated, very much more so on the right than on the left. The cuticle is distinctly striated. The cervical papillae are slightly asymmetrical, the left being a little in front of the right, at a distance of 0·07–0·074 mm. from the head in the male and 0·08–0·09 mm. in the female. The nerve ring and the excretory pore are at 0·18–0·20 mm. and 0·24–0·26 mm. from the anterior tip in the male and 0·24–0·26 mm. and 0·28–0·32 mm. in the female.

The mouth is surrounded by two pairs of lips, one lateral and the other dorso-ventral. The lateral pair possesses three pairs of teeth. Each of the pseudolips has three minute papillae near its margin and a pair of submedian papillae, with a laterally situated amphid near its base. Each dorso-ventral "lip" is also provided with a pair of submedian papillae. The dorso-ventral "lips" with a pair of sabre-shaped chitinous plates, on either side, give the mouth cavity a peculiar shape in the end-on-view, the two lateral chitinous plates each arising from the base of the dorsal and the ventral lip, and the two inflations, one on each lateral side are also observed. The buccal cavity is 43–45μ long and 30–32μ wide in the male and 52–58μ long by 36–38μ wide in the female. The two portions of the oesophagus measure 0·51–0·53 mm. and 2·88–2·92 mm. and 0·56–0·61 mm. and 3·16–3·91 mm. in length, respectively in the male and female. The entire oesophagus, 3·4–3·5 mm. long in the male and 3·7–4·5 mm. in the female occupies $\frac{1}{3}$ and $\frac{1}{4}$ of the body length in the two respective sexes.

Male. The males are smaller and thinner than the females, measuring 10·2–11·9 mm. in length. The anterior half of the body is thinner than the posterior half, the average width of the two portions being 0·16 mm. and 0·26 mm. respectively. The tail is enclosed in well developed, broad but asymmetrical caudal alae, the left being much broader than the right, measuring 0·13 mm. and 0·07 mm. respectively in width and 0·56 mm. and 0·49 mm. in length. The cloaca opens at a distance of 0·21 mm. from the tip of the tail. There are four pairs of long rib-like papillae, precloacal in position and another two pairs, of the same unusual type, of long postcloacal papillae. The first and the third precloacals of the left side are almost of the same length as that of the alae, measuring 0·13 mm. and 0·14 mm. in length respectively. The second precloacal on the left measures 0·11 mm. and the fourth is 0·058 mm. long. The first and the second papillae on the right, extend up to the margin of the ala, being 0·079 mm. long. The third and fourth, respectively decrease in length. The first and second pair of postcloacals are also fairly long, measuring 0·067 mm. in length. There are two groups of sessile papillae. Midway between the second pair of postanal papillae and the tip of the tail there is a group of papillae concentrically arranged, numbering eight pairs. A pair of papillae, somewhat larger in size is situated slightly in front of the first pair of this ring of papillae. All these are arranged on a slightly raised pad-like structure. Another group of papillae numbering three—a single and a pair—are located just near the tip of the tail. On either side of the unpaired papillae a pair of phasmids is also observed. The spicules are very unequal, the two measuring 0·62–0·69 mm. and 0·093–0·16 mm. The right spicule is short, thick and navicular in shape, whereas the left is thin and acicular. The gubernaculum has a short base, but wide and deeply notched anterior free edges.

Female. The females are larger than the males measuring 12·9–16·1 mm. in length. The difference in thickness of the anterior and the posterior halves of the body is more marked than in males.

The average width of the anterior and the thinner half is ·18 mm. and that of the posterior is ·28 mm. The vulva is flush with the body and opens equatorially, about 5 mm. from the tip of the tail. The eggs are thick shelled and embryonated, 0·03–0·037 by 0·38–0·40 mm. long. The tail is 0·185–0·21 mm. long and possesses a cluster of cuticular processes, at its tip.

Host : *Upupa epops*
Habitat : Small intestine
Locality : Hyderabad, India.

Summary

1. The writer has reviewed the literature on the systematics of genera *Habronema, Excisa, Cyrnea* and *Sicarius*. The genus *Habronema* is divided into two subgenera, *Habronema* and *Aviabronema*, for the parasites of mammals and birds respectively. A key to the known species of the genus is given.

2. Eight new species* of *Aviabronema* are described; of these six are reported from birds of prey, one from the green pigeon (*Crocopus phoenicopterus*) and the other from the grackle (*Gracula intermedia*).

3. A new species of *Excisa* and another new species of *Cyrnea* are described; *Sicarius dipterum* is reported from India.

Acknowledgment

The writer takes this opportunity of expressing his deep gratitude to The Nuffield Foundation and The Royal Society for the award of bursary, enabling him to carry out the work at the London School of Hygiene and Tropical Medicine, as a result of which the present paper is produced. The writer is profoundly indebted to Professor J. J. C. Buckley, for inspiring suggestions, discussions and guidance. He is also thankful to the Library staff for assistance with the literature.

Gratitude is also due to Professor S. N. Singh of the Osmania University whose helpful criticisms and suggestions have always been of immense help.

References

Baylis, H. A., 1931.—"On a nematode parasite of Pangolins." *Ann. Mag. nat. Hist.*, **8**, 191–194. (W.L. 1050)

————, 1934.—"On a collection of Cestodes and Nematodes from small Mammals in Tanganyika territory." *Ibid.*, **13**, 338–353.

————, 1939.—The fauna of British India. Nematoda, Vol. II. Taylor and Francis Ltd., London.

Baylis, H. A. and Daubney, R., 1926.—A Synopsis of the families and genera of Nematoda. pp. 277. London.

Besseru, B., 1955.—"On some Nematodes from African Birds." *Ann. Mag. nat. Hist.*, **8**, 765–786. (W.L. 1050)

Chabaud, A. G., 1955.—"Remarques sur la symétrie céphalique des nématodes et hypothéses concernant l'evolution de cette symétrie chez les phasmidiens parasites." *Bull. Soc. zool. Fr.*, **80**, 314–323. (W.L. 5401)

————, 1957.—"Nématodes parasites d'oiseaux en Tunisie. Collection C. Vermeil." *Arch. Inst. Pasteur.*, **34**, 155–166. (W.L. 1843).

————, 1958.—"Essai de classification des nématodes Habronematinae." *Ann. Parasit. hum. comp.*, **33**, 445–508. (W.L. 899a)

*Type specimens of the species described in this paper are deposited in the Department of Parasitology of the London School of Hygiene and Tropical Medicine.

Chabaud, A. G. and Brygoo, E. R., 1958.—"Sur un nouveau nematode Habronème parasite de Rapaces à Madagascar." *Mem. Inst. sci. Madagascar*, **12**, 127–138. (W.L. 132516).

Chabaud, A. G. and Rousselot, R., 1956.—"Un nouveau spiruride parasite du gorille *Chitwoodspirura wehri* n.g., n.sp.", *Bull. Soc. Pat. exot.*, **49**, 467–472. (W.L. 5310)

————, 1957.—"Description d'un nematode : *Cyrnea* (*Cyrnea*) *antennifera* n.sp., interessant par ses caractères cèphaliques." *Bull. Soc. zool.*, **82**, 420–429. (W.L. 5401).

Chandler, A. G., 1941.—"A new Spiruroid Nematode *Habronema americanum* from the Broadwinged Hawk *Buteo platypterus*." *Parasit.*, **27**, 184–185. (W.L. 11428).

Chitwood, B. G. and Chitwood, M. B., 1950.—An introduction to Nematology. Section 1., Anatomy, Baltimore, U.S.A.

———— and Wehr, E. E., 1934.—"The value of cephalic structures as characters in Nematode classifications, with special reference to the super-family Spiruroidea." *Z. Parasitenk.*, **7**, 274–335. (W.L. 23536b)

Cram, E. B., 1927.—"Bird parasites of the Nematode suborders Strongylata, Ascaridata and Spirurata." *Bull. U.S. nat. Mus.*, **140**, (W.L.5659)

Dollfus, R. Ph. and Chabaud, A. G., 1957.—"Phènomènes de convergence chez les spirurides en particulier dans les sous-familles Habronematinae Chitwood et Wehr 1932 et Schistorophinae L. Travassos 1918., leur importance pour une classification naturelle des spirurides (Nematoda)." *Bull. Soc. zool. Fr.*, **82**, 88–102. (W.L. 5401)

Ezzat, Mohammed A. E., 1945.—"Helminth parasites of some ungulates from the Giza Zoological Gardens. With an appendix on some Nematodes from the African Rhinoceros." *Bull. Minist. Agric. Egypt*, **241**, 1–104. (W.L. 45750).

Fedyushin, A. V., 1946—"A new Nematode *Cyrnea lyruri* n.sp." *Gelmintologitcheskii shornik*. Moscow. 267–273.

Foster, A. O. and Chitwood, B. G., 1937.—"A new Nematode *Habronema clarki* n.sp. from *Hydrochoerus isthmius* Goldman." *Proc. helminth. Soc. Wash.*, **4**, (W.L. 16747a)

Freitas, J. F. T., and Lent, H., 1947.—"Spiruroidea parasites de 'Rheiformes' (Nematoda)." *Mem. Inst. Osw. Cruz.*, **45**, 743–760. (W.L. 134651)

Inglis, W. G., 1954.—"On some Nematodes from Indian Vertebrates. 1. Birds." *Ann. Mag. nat. Hist.*, **7**, 821. (W.L. 1050)

Johnston, H. and Mawson, P. M., 1941a.—"Some Nematodes from Australian Birds of Prey." *Trans. roy. Soc. S. Aust.*, **65**, 30–35. (W.L. 21654)

————1941b.—"Some Nematode parasites of Australian Birds." *Proc. Linn. Soc. N.S.W.*, **66**, 250–256. (W.L. 16790a)

————, 1941c.—"Additional Nematodes from Australian Birds." *Trans. roy. Soc. S. Aust.* **65**, 254–262. (W.L. 21654)

Kung, C. C., 1948.—"On Some new species of Spirurids from terrestrial vertebrates with notes on *Habronema mansioni*, *Physaloptera paradoxa* and *Hartertia zuluensis*. *J. Helminth.*, **22**, 141–164. (W.L. 11224c)

Li, H. C., 1934.—"Report on a collection of parasitic Nematodes, mainly from North China, Part II. Spiruroidea." *Trans. Amer. micr. Soc.* **53**, 174–195. (W.L. 21400)

Maplestone, P. A., 1929.—"Two new species of Nematodes from Indian Hosts." *Rec. Indian. Mus.*, **31**, 87–92. (W.L. 17746)

————, 1930.—"Parasitic Nematodes obtained from animals dying in the Calcutta Zoological Gardens." 1–3. *Ibid.* **32**, 385–412.

————, 1932.—"Parasitic Nematodes obtained from animals dying in the Calcutta Zoological Gardens." 9–11. *Ibid.* **34**, 229–261 (W.L. 17746)

MIRZA, M. B., and BASIR, M. A., 1938.—"On a collection of Nematodes from Hyderabad Deccan." *Z. Parasitenk.*, **10,** 216–220. (W.L. 23536b).

NEVEU-LEMAIRE, M., 1927.—"*Protospirura hamospiculata* n.sp., nématode parasite d'un pangolin africain, *Manis* (*Pholidotus*) *temmincki*." *Ann. Parasit. hum. comp.*, **5,** 107–113. (W.L. 899a)

ORTLEPP, R. J., 1925.—"On two Nematode parasites from the gizzard of Peafowls." *J. Helminth.*, **3,** 177–184. (W.L. 11224c)

————, 1934.—"On *Habronema murrayi* n.sp. from the barn owl-*Tyto alba*." *Onderspoort J. vet. Sci.*, **5,** 351–355. (W.L.15681a)

————, 1938.—"South African Helminths Part V. Some Avian and Mammalian Helminths." *Ibid.*, **11,** 63–104.

POPOWA, K. A., 1927.—"Ein neuer Nematoda des Wiedehopfes (*Upupa epops*) *Habronema dipterum* n.sp. (en russe résuméen allemand)." *Sammlung Helminth. Arbeiten K.I. Skrjabin gewidmet.* Moscow, 180–185.

SANDGROUND, J. H., 1929.—"Some new parasitic Nematodes from Yucatan, (Mexico), including a new genus of strongyle from cattle." *Bull. Mus. comp. Zool. Harv.*, **69,** 515–524. (W.L. 4604).

SANWAL, K. C., 1951.—"On a new Avian nematode, *Habronema thapari* n.sp. (sub. fam. spirurinae, Railliet & Henry, 1915) from the blue jay *Coracias benghalensis* L.. *Indian J. Helminth.*, **3,** 76–86. (W.L. 9939g)

SARWAR, M. M., 1954.—"On the transference of *Parabronema skrjabini* Rassowska, 1924 to *Squamonema* Thiel, 1925 and its occurrence in sheep and goats in Indo-Pakistan." *J. Helminth.*, **20,** 143–150. (W.L. 11224c)

SKRJABIN, K. I., 1941.—"On the rearrangement of the taxonomy of nematodes of the Acuariidae and Ancyracanthidae families." *C. R. Acad. Sc. U.R.S.S.* **30,** 470–473. (W.L. 7450)

TSCHERNIKOWA, C., 1934.—"Un nouveau nématode : *Habronema skrjabini* n.sp. du chat sauvage." *Ann. Parasit. hum. comp.*, **12,** 29–34. (W.L. 899a)

VAZ., Z., 1936.—"Estudos söbre nematoides parasitas da ema (*Rhea americana*). *Arch. Inst. biol. S. Paulo*, **7,** 253–266. (W.L. 1965c)

VUYLSTEKE, C., 1936.—"Un nématode nouveau du pangolin d'Afrique." *Rev. Zool. Bot. Afric.* **29,** 13–15. (W.L. 19322).

————, 1937.—"*Habronema congolense* n. nom. (Nematode). Note synonymique." *Ibid.*, **30,** 140.

————, 1953.—"Nematodes parasites d'oiseaux". *Exploration du Parc National de l'Upemba. Mission G. F. De Witte* (1946-49). *Brussels, Fasc.* **17,** 1–41.

WALTON, A. C., 1923.—"Some new and little known Nematodes." *J. Parasit.*, **10,** 59–70. (W.L. 11482)

————, 1928.—"A revision of the Nematodes of the Leidy Collection." *Proc. Acad. nat. Sci. Philad.*, **79,** 49–163. (W.L. 16590)

WEHR, E. E., 1931.—"A new species of Nematode worm from the Sage Grouse." *Proc. U.S. nat. Mus.* **79,** 1–3. (W.L. 16944)

————, 1933.—"A new Nematode from the Rhea." *Ibid.*, **82,** 1–5.

YAMAGUTI, S., 1935.—"Studies on the Helminth Fauna of Japan. Part 12. Avian Nematodes, I." *Jap. J. Zool.*, **6,** 403–431. (W.L. 10881w)

————, 1941.—"Studies on the Helminth Fauna of Japan. Part 36. Avian Nematodes, II." *Ibid.*, **9,** 441–480.

YORKE, W. and MAPLESTONE, P. A., 1926.—The Nematode parasites of Vertebrates. J. and A. Churchill, London.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, pp. 49–60.

On Some New Species of *Physaloptera* from Indian Birds

By Syed Mehdi Ali, M.Sc., Ph.D.
Department of Zoology, College of Science, Osmania University, Hyderabad, India

Physalopteridae Leiper, 1908

Physalopterinae Railliet, 1893

Physaloptera Rudolphi, 1819

In view of Morgan's (1943) contribution "The Physalopterinae (Nematode) of Aves", it is unnecessary here to review the literature in this group; but it should be noted that Morgan regards *Physaloptera alata* as having four pairs of pedunculated papillae on the male tail, whereas Cram (1927), reproducing Rudolphi's (1819) and Seurat's (1914) descriptions, states the number to be five. Ortlepp (1922), Li (1934) and Singh (1949) also described the number as five.

Physaloptera alata Rudolphi, 1819

This species was collected from *Milvus migrans govinda*, the common pariah kite of India. The material for study consists of both males and females. The measurements are as follows:

Male: Body length, 17·9–18·9 mm.; maximum diameter, 0·50–0·54 mm.; nerve ring to anterior end 0·27–0·34 mm.; cervical papillae to anterior end 0·31–0·38 mm.; excretory pore to head end 0·60–0·68 mm.; oesophagus, muscular portion 0·40–0·48 mm.; glandular portion 2.77–3.46 mm.; spicules 0·44–0.48 mm.; glandular portion 2.77–3.46 mm.; spicules 0·44–0·48 mm. and 0·48–0·52 mm.; tail 0·59–0·68 mm.; Caudal alae length 1·38–1·47 mm.; breadth 0·82–0·88 mm.; caudal papillae, five pairs of pedunculated papillae of which two pairs are precloacal, one adanal and two pairs postcloacal; sessile papillae; three precloacal, two pairs immediately postcloacal, one pair midventral between the second pedunculated postcloacals, one pair midway between the last pedunculated postcloacal and tip of the tail.

Female : Body length 18·6–21·21 mm.; maximum body width 0·63–0·69 mm.; nerve ring from head end 0·38–0·42 mm.; cervical papillae from anterior tip 0·49–0·52 mm.; excretory pore 0·72–0·80 mm.; oesophagus, muscular portion 0·50–0·59 mm., glandular portion 3·52–3·96 mm.; vulva to anterior end 4·2–4·73 mm.; vagina length 1·95–2·04 mm.; eggs, 31–33$\mu \times$16–19μ ; tail 0·38–0·42 mm.

Host : *Milvus migrans govinda*

Habitat : Gizzard

Locality : Hyderabad, India

Physaloptera alata chevreuxi Seurat, 1914.

The pale harrier, *Circus macrourus*, was found infected with a pair of this species. The male and the female were collected from the oesophagus and the gizzard respectively of the host. The principal measurements are as follows :

Male : Body length 15·6 mm.; maximum diameter 0·54 mm.; nerve ring to anterior end 0·27 mm.; cervical papillae to head end 0·26 mm.; excretory pore from anterior end 0·56 mm.; oesophagus, muscular 0·45 mm., glandular 2·1 mm.; spicules, right 0·56 mm., left 0·50 mm.; tail, 0·82 mm.; caudal alae length 1·49 mm., maximum breadth 0·75 mm.; caudal papillae, five pairs pedunculated ; sessile, two pairs immediately behind cloaca, two pairs equidistant on tail, three immediately precloacal.

Female : Body length 18·5 mm.; maximum body width 0·74 mm.; nerve ring to head end 0·39 mm.; excretory pore 0·64 mm.; cervical papillae from anterior end 0·54 mm.; oesophagus, muscular 0·49 mm.; glandular 2·86 mm.; vulva to anterior end 4.82 mm.; vagina 1·98 mm.; eggs, 28–33$\mu \times$15—18μ ; tail 0·48 mm.

Host : *Circus macrourus*

Habitat : Oesophagus

Locality : Hyderabad, India

Physaloptera indiana n.sp.

Numerous males and females of this species were collected from oesophagus and gizzard of the buzzard eagle, *Butastur teesa*.

These worms are fairly stout, having a thick and transversely striated cuticle. The anterior end is almost covered by a prepuce-like sheath of the cuticle. The mouth is surrounded by two well developed lips, each provided with three teeth, of which the outer two are less prominent than the inner one. Each lip carries a pair of cephalic papillae and an amphid. The shape of the cuticle forming the prepuce varies from one to the other member of the same species, hence of no taxonomic value. The nerve ring surrounds the muscular portion of the oesophagus at a distance of 0·29–0·32 mm. from the head end in the male and 0·36–0·42 mm. in the female. The excretory pore opens at 0·74–0·80 mm. from the anterior tip in the male and 0·81–0·86 mm. in the female. The cervical papillae are located slightly behind the nerve ring at 0·36–0·39 mm. and 0·46–0·54 mm. from the anterior end in the two sexes respectively. The oesophagus measures 3·5–3·9 mm., and 4·3–4·9 mm. long respectively in the male and the female. It occupies about $^1/_5$ of the body length in both sexes.

Male : The males are 19–21 mm. long, having a maximum breadth of 0·52–0·55 mm. The diameter of the head is 0·18–0·19 mm. The sharp tapering tail is 0·82–0·94 mm. long and is enclosed by caudal alae measuring 1·62–1·78 mm. in length and 0·73–0·78 mm. in maximum expansion. The alae do not meet each other ventrally. There are altogether five pairs of pedunculated papillae of which two pairs are precloacal, one adanal and the rest postcloacal. The first postcloacal pair is much smaller than the rest. The first four pairs are closely set together, while the fifth is somewhat further from the fourth pair. There are four pairs of sessile papillae. Of these the first pair is precloacal, the second and the third pairs form a group immediately behind the cloaca and the fourth pair is in between the fourth and the fifth pedunculated pairs. The spicules are similar in shape, but unequal in length, being 0·67–0·72 mm. and 0·39–0·42 mm. respectively.

Female : The female measures 21·6–24·5 mm. in length and 0·57–0·62 mm. in maximum width. The head diameter is 0·20–0·22 mm. The vulva opens at 6·76–7·2 mm. from the head and almost at the junction of the 1st and the 2nd third of the body. The vagina is muscular and 0·14–0·19 mm. long and the common trunk of the uterus 1·8–2 mm. long. The uterus is full of embryonated eggs measuring 0·027–0·031 mm. in length and 0·014 0·016 mm. in breadth. The tail is short, measuring 0·46–0·5 mm. in length.

Relationships : In possessing unequal spicules this species resembles *P. acuticauda* Molin, 1860, and *P. mexicana* Caballero, 1937.

Caballero had described *P. mexicana* as having five pairs of stalked papillae, 3 pairs of subventral sessile genital papillae and moderately unequal spicules, 0·334 mm. and 0·43–0·46 mm. long (a ratio of 1 : 1·3–1·4). Morgan (1943) examined the "cotypes" and published his results as, "spicules very unequal, the ratio between the length of the right and the left ones being about 1 : 2·5," however his figure agrees with that of Caballero as to the number and location of papillae. Lucker (1956) re-examined the type and the "cotype" specimens and agreed with Morgan as far as the length and ratio of the spicules was concerned, but found "thirteen ventral and subventral sensory organs" instead of six.

The present worm does not conform with the worm described by Caballero, Morgan and Lucker in one character or the other. It is similar to Morgan's and Lucker's in the ratio of the spicules, but differs in their lengths (left spicule 0·67–0·72 mm., right spicule 0·39–0·42 mm.), being much smaller. The length of the spicules is nearer to that described by Caballero, but it differs in ratio. The number of sessile papillae is the same (6) as described by Caballero, but the arrangement is quite different. While a single sessile pair of papillae is reported as posterior to the cloaca in Caballero's worm, there are two such pairs in the present species. The number of sessile papillae described by Lucker is thirteen. The single sessile papilla in front of the cloaca reported by Lucker is absent in the present worm, nor are there any papillae on the tail region behind the last pedunculated pair. Moreover the approximation of the first two pairs of pedunculated papillae shown in Lucker's figure is not to be found in the worm under discussion. The first postanal pedunculated pair is much smaller than the other pairs. The inflated precloacal portion of the bursa was not observed.

Physaloptera spp.
Heads and end-on views.

P. alata Rudolphi, 1819.
Fig. 1.—Lateral view. Fig. 2.—Ventral view. Fig. 3.—End-on view.

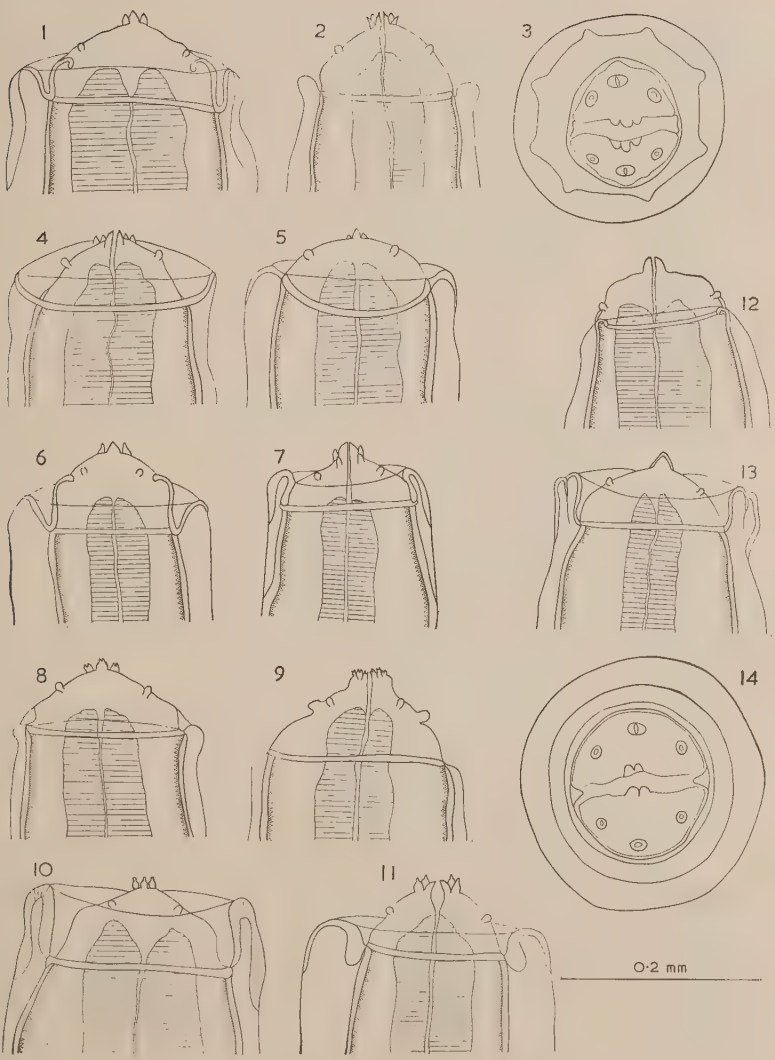
P. alata chevreuxi Seurat, 1914.
Fig. 4.—Lateral view. Fig. 5.—Ventral view.

P. indiana n.sp.
Fig. 6.—Lateral view. Fig. 7.—Dorsal view.

P. microcauda n.sp.
Fig. 8.—Lateral view. Fig. 9.—Dorsal view.

P. sultanae n.sp.
Fig. 10.—Lateral view. Fig. 11.—Dorsal view.

P. buckleyi n.sp.
Fig. 12.—Lateral view. Fig. 13.—Dorsal view. Fig. 14.—End-on view.


1
2
3
4
5
12
6
7
13
8
9
14
10
11
0·2 mm

P. acuticauda differs in having four pairs of pedunculated papillae, much longer spicules, bigger eggs, with a uterus of different type (c) and with a larger number of sessile papillae.

In view of these differences, the present worm is regarded as new and named as *P. indiana.*

Host : *Butastur teesa.*

Habitat : Oesophagus and gizzard.

Locality : Hyderabad, India.

Physaloptera microcauda n.sp.

A single pair of this parasite was recovered from the oesophagus of the Indian white-backed vulture, *Pseudogyps benghalensis.* These are rather slender worms with thick cuticle having transverse striations. The male and the female measure 18·3 mm. and 20 mm. respectively in length and 0·32 mm. and 0·36 mm. in maximum diameter. The nerve ring and the excretory pore, are 0·31 mm. and 0·62 mm. respectively from the head end in the male and 0·38 mm. and 0·66 mm. in the female. The cervical papillae are 0·4 mm. and 0·43 mm. from the anterior tip in the male and the female respectively. There are three teeth on each lip and each one seems to have bifid tips. Two pairs of cephalic papillae and a pair of amphids are also present. The oesophagus is 3·35 mm. long in the male and 4·18 mm. in the female. The muscular portion of the oesophagus is 0·48 mm. and 0·59 mm. in the male and female respectively.

Male : The males have a comparatively shorter tail (0·54 mm.) and smaller but well expanded alae measuring 1·1 mm. in length and 0·56 mm. in maximum breadth. Each caudal ala seems to be almost divided into two halves by a deep notch ; they meet ventrally at their proximal ends. There are five pairs of pedunculated papillae arranged in three groups. The first group is of two pairs of preanals, the second group consists of two pairs of postanals and the third group has a single pair almost midway between the last pair and the tip of the tail. Immediately in front of the cloaca there are three sessile papillae. Two more pairs of such papillae are located mid-ventrally on the tail. The spicules are almost equal being 0.83 mm. and 0·80 mm. respectively.

Female : The females are slightly longer than the males. The vulva is flush with the body surface, 4·4 mm. from the anterior end.

The vagina is long and muscular, measuring 1·98 mm. The eggs are embryonated, 37–38 μ long and 16–18μ wide. The tail measures 0·55 mm. and is comparatively longer than those of the other species described herein.

Relationships : In not possessing the postcloacal sessile papillae, the present worm resembles *P. brachycerca* Kries, 1938, but has two pairs of postanal sessile papillae instead of one pair as in the latter. The shape of the caudal alae—with a deep constriction in the mid-length—the disposition of the caudal papillae and other body measurements differ in the two. Moreover the alae meet anteriorly. Hence it is regarded as a new species and named *P. microcauda* n.sp.

Host : *Pseudogyps benghalensis*

Habitat : Gizzard

Locality : Hyderabad, India.

Physaloptera sultanae n.sp.

Several males and females of this species were available for study. The worms were collected from the gizzard of the Pallas' fishing eagle, *Haliaëtus leucoryphus*.

These stout worms have a thick cuticle with fine transverse striations. The male and female respectively measure 18·9–19·2 mm. and 20·3–23·3 mm. in length and 0·49–0·55 mm. and 0·78–0·85 mm. in maximum diameter. The lips are well developed, each bearing three teeth of equal size with a knob-like tip. Each lip possesses a pair of submedian papillae and a laterally situated amphid. The prepuce-like cuticular collar is well developed and reflects over the head almost concealing it. The lateral fields are moderately wide. The cervical papillae project laterally at 0·32-0·36 mm. from the head end in the male and 0·36–0·38 mm. in the female. The nerve ring and the excretory pore are 0·26–0·29 mm. and 0·56–0·58 mm. respectively from the anterior end in the male and 0·27–0·30 mm. and 0·58–0.60 mm. in the female. The oesophagus occupies $^1/_6$ of the body length in both the sexes, being 3·42–3·50 mm. and 3·86–4·6 mm. long in the male and the female respectively. The muscular and the glandular portions measure 0·54–0·58 mm. and 2·9–3·0 mm. respectively in the male and 0·75–0·79 mm. and 3·1–3·9 mm. respectively in the female.

Male : The tail is 0.88 mm. long and bears long and wide caudal alae, measuring 1·94–2·3 mm. long and 0·44 mm. wide, the left ala being broader than the right. There are five pairs of pedunculated papillae of which two are preanal, one adanal and two postanal in position. Three sessile papillae are to be seen in front of the cloaca and two pairs of the same type immediately posterior to it. Another pair of papillae asymmetrically arranged is found between the first pedunculated postanal pair of the papillae. The spicules are small and almost equal in length measuring 0·41 mm. and 0·44 mm.

Female : The conical but somewhat bluntly pointed tail is 0·47 mm. long. The vulva opens at the junction of the 1st and the 2nd third of the body at 7·0–7·78 mm. from the head end. The muscular vagina measures 1·86–2·3 mm. in length. The uterus and its ducts are tightly packed with embryonated eggs, 0·038–0·04 mm. long by 0·17–0·19 mm. broad.

Relationships : The present species does not agree with any of the known species in having just three postanal sessile papillae, of which two pairs are immediately behind the cloaca and one asymmetrical pair between the last two pairs of pedunculated papillae. In the number of the sessile and the pedunculated papillae it resembles *P. indiana* reported in this communication, but the spicules and the disposition of the papillae differ in the two. In *P. indiana* the spicules are unequal as against the equal spicules in the present worm. In view of the characters discussed, it is regarded as a new species. It is proposed to name it *P. sultanae* after by student, Ameer Sultana.

Host : *Haliaëtus leucorhyphus*

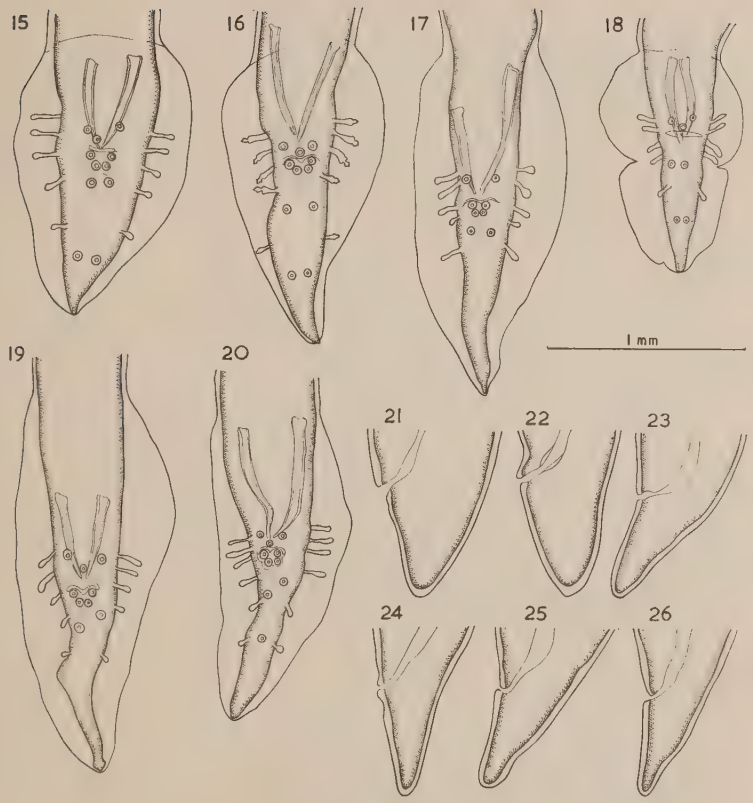
Habitat : Gizzard

Locality : Hyderabad, India.

Physaloptera buckleyi n.sp.

Two male and two female *Physaloptera* were collected on two occasions from the oesophagus and gizzard of the short-toed eagle, *Circaëtus gallicus*.

These are fairly stout worms having a thick cuticle which is transversely striated. In both the sexes the extremities are attenuated. The mouth is surrounded by well developed lateral lips, each provided with teeth—one large and the other smaller—and with a pair of cephalic papillae. An amphid is also present on either lip. The cuticle



Physaloptera spp.
Caudal regions of males, ventral view, and of females, lateral view.

Fig. 15.—*P. alata* Rudolphi, 1819. Fig. 16.—*P. alata chevreuxi* Seurat, 1914. Fig. 17.—*P. indiana* n.sp. Fig. 18.—*P. microcauda* n.sp. Fig. 19.—*P. sultanae* n.sp. Fig. 20.—*P. buckleyi* n.sp. Fig. 21.—*P. alata* Rudolphi, 1819. Fig. 22.—*P. alata chevreuxi* Seurat, 1914. Fig. 23.—*P. indiana* n.sp. Fig. 24.—*P. microcauda* n.sp. Fig. 25.—*P. sultanae* n.sp. Fig. 26.—*P. buckleyi* n.sp.

in the anterior portion forms a collar round the head. The nerve ring surrounds the muscular portion of the oesophagus at a distance of 0·39–0·41 mm. from the head end in the male and 0·47–0·49 mm. in the female. The cervical papillae are just posterior to the nerve ring. The excretory pore opens at 0·50–0·56 mm. and 0·54–0·58 mm. from the anterior end in the male and female respectively. The oesophagus occupies $^1/_5$th and $^1/_6$th of the total body length in the male and the female respectively. Its anterior and posterior portions measure 0·48–0·52 mm. and 2·81–3·12 mm. in length in the male and 0·56–0·59 mm. and 2·86–3·17 mm. in the female.

Male : The males are not markedly smaller than the females, measuring 16·4–17·9 mm. in length and 0·54–0·6 mm. in maximum width. The conical tail which is 0·81–0·86 mm. long, is provided with six pairs of pedunculated papillae and ten sessile papillae. The two anteriormost pairs of pedunculated papillae are preanal, the third is adanal and the remainder postanal in position. Of the sessile papillae, three are just in front of the cloaca and two pairs immediately posterior to it. A pair of sessile papillae, asymmetrical in position is seen between the first and the second pairs of postanal pedunculated papillae. There is yet another single papilla, midventrally situated, between the last pair of pedunculated ones. The spicules are slightly subequal, being 0·58 mm. and 0·63 mm. long. The caudal alae are broad and long, measuring 1·49–1·54 mm. in length, with a maximum expansion of 0·73–0·86 mm. The two alae do not meet anteriorly.

Female : The females are 18·3–19·2 mm. long with a maximum diameter of 0·61–0·63 mm. The tail is small, stumpy and measures 0·44 mm. in length. The vulva opens at a distance of 5·10–5·19 mm. from the head end. The vaginal length is 1·54–1·68 mm. The ovaries are located in the posterior portion of the body lying one behind the other and measure 0·032–0·036 mm. long and 0·015–0·016 mm. wide.

Relationships : This species differs from all the known species of avian *Physaloptera* in possessing six pairs of pedunculated papillae, a character which is itself sufficient to distinguish it as a new species. In having an unpaired papilla on the tail between the last pedunculated pair, it resembles *P. crosi* Seurat, 1914, but differs from it in the length and proportion of oesophagus to body length, and in having subequal spicules of greater length and in other measurements. It is therefore regarded as a new species and named after Professor J. J. C. Buckley as *Physaloptera buckleyi* n.sp.

Host : *Circaëtus gallicus*
Habitat : Oesophagus and gizzard
Locality : Hyderabad, India.

Type specimens of the species described in this communication are deposited in the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Key to Species of *Physaloptera* in Birds

1. Female didelphic—A type 2
   Female didelphic—B type .....*P. acuticauda* Molin, 1860
2. Males with 4 pairs of pedunculated papillae, caudal alae narrow, sessile papillae thirteen (3 precloacal, 5 pairs postcloacal.....*P. losseni* Ortlepp, 1937
   Males with 5 pairs of pedunculated papillae..... 3
   Males with 6 pairs of pedunculated and 10 pairs of sessile papillae..... *P. buckleyi* n.sp.
3. Males with immediate postcloacal sessile papillae absent 4
   Males with immediate postcloacal sessile papillae present 5
4. Caudal alae with five sessile papillae (3 preanal, 1 pair postanal .....*P. brachycerca* Kreis, 1938
   Caudal alae with seven sessile papillae (3 preanal, 2 pairs postanal).....*P. microcauda* n.sp.
5. Males with unequal spicules..... 6
   Males with equal or sub-equal spicules..... 8
6. Caudal alae with eight sessile papillae (2 precloacal, 3 pairs postcloacal..... *P. indiana* n.sp.
   Caudal alae with more than ten sessile papillae..... 7
7. Caudal alae with thirteen sessile papillae (3 precloacal, 5 pairs postcloacal)... *P. mexicana* (Caballero, 1937) Lucker, 1956
   Caudal alae with twenty-three sessile papillae (3 precloacal, 10 pairs postcloacal.....*P. reevisi* Chu, 1931
8. Caudal alae with nine sessile papillae (3 precloacal, 3 pairs postcloacal..... *P. sultanae* n.sp.
   Caudal alae with eleven sessile papillae..... 9
   Caudal alae with more than twelve sessile papillae..... 10
9. Vulva near the end of oesophagus...*P. alata* Rudolphi, 1819
   Vulva a little anterior to the middle of the body..... ..... *P. galinieri* Seurat, 1914
10. Caudal alae with thirteen sessile papillae (3 precloacal, 5 pairs postcloacal) ..... *P. rapacis* Mönnig, 1926
    Caudal alae with fourteen sessile papillae (3 precloacal, 4 pairs postcloacal and 1 unpaired... *P. crosi* Seurat, 1914

Acknowledgment

The production of the present paper at the London School of Hygiene and Tropical Medicine was made possible by the financial assistance from the Royal Society and Nuffield Foundation, for the award of which the writer wishes to express his deep gratitude.

References

Baylis, H. A., 1939.—The fauna of British India, including Ceylon and Burma. Nematoda, Vol. 2, London.

Caballero, C. E., 1937.—"Contribución al conocimiento de los nemátodos de las aves de Mexico. 11." *Rev. Med. trop. Fabance.* **3,** 25–35 (W.L.18902a).

Chu, H., 1931.—"Nematodes from flying lemurs in the Philippines and from birds in China." *J. Parasit.*, **17,** 155–160. (W.L.11428).

Cram, E., 1927.—"Bird parasites of the nematode suborders Strongylata, Ascaridata and Spirurata." *Bull. U.S. nat. Mus.*, **140,** 1–465. (W.L. 5659).

Johnston, T. and Mawson, P., 1941.—"Some Nematodes from Australian birds of Prey." *Trans. Roy. Soc. S. Australia*, **65,** 30–35. (W.L.21654).

Kreis, H., 1938.—"Parasitische Nematoden der schweizerischen wissenschaftlichen Expedition nach Angola (Afrika) in Jahre 1932." *Zentr. Bakt., I. Abt.* Orig. **142,** 90–105. (W.L.23684).

Li, H. 1934.—"Report on a collection of parasitic nematodes mainly from North China. 11 Spiruroidea." *Trans. Amer. Micro. Soc.*, **53,** 174–195. (W.L. 21400a).

Lucker, J. T., 1956.—"The Structure, Synonyms and Hosts of *Physaloptera mexicana* (Nematoda : Physalopteridae)." *Proc. Helm. Soc. Wash.*, **23,** 1–4. (W.L.16747a).

Morgan, B. B., 1940.—"The Physalopterinae (Nematoda) of North America." *J. Parasit.*, **26,** 40. (W.L.11428).

————, 1941.—"A summary of the Physalopterinae (Nematoda) of North America." *Proc. Helm. Soc. Wash.*, **8,** 28–30. (W.L.16747a).

————, 1941,—"Additional Notes on North American Physalopterinae (Nematoda)." *Ibid.*, 63–64.

————, 1943.—"The Physalopterinae (Nematoda) of Aves." *Trans. Amer. Micro. Soc.*, **62,** 72–80. (W.L.21400a).

Ortlepp, R., 1922.—"The Nematode genus *Physaloptera* Rud." *Proc. Zool. Soc. Lond.*, 999–1107. (W.L.16969b).

————, 1936,—"Some undescribed species of the Nematode genus *Physaloptera* together with a key to the sufficiently known forms." *Onderspoort J. vet. Sci.*, **9,** 71–84. (W.L.15681a).

Schulz, R., 1927.—"Die Familie Physalopteridae Leiper, 1908, Nematodes und die principen ihre Klassifikation." *Samml. Helm. arb.* Prof. K. I. Skrjabin, 287–312.

Singh, S. N., 1949.—"Studies on the Helminth Parasites of Birds in Hyderabad State. Nematoda III". *J. Helminth.*, **23,** 34–35. (11224c).

Yamaguti, S., 1941.—"Studies on helminth fauna of Japan. 36. Avian Nematodes II." *Jap. J. Zool.*, **9,** 442–480. (W.L.10881).

Yorke, W. and Maplestone, P., 1926.—Nematode Parasites of Vertebrates, London.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 61–68.

On Two Capillariid Nematodes Parasitic in British Water Shrews (*Neomys fodiens* Schr.)

By J. Bernard
State Entomological Research Station, Gembloux, Belgium

Several years ago, thanks to a grant obtained from the British Council, I spent a fortnight in Oxford at the Bureau of Animal Population. During this stay, I was able to trap some small mammals in a wood near Oxford. Among these mammals were several water shrews, *Neomys fodiens* Schr., which were brought to Belgium to be put in the collection of the State Entomological Research Station. Later, being interested in parasitic helminths of little mammals, I dissected the shrews in order to collect their parasites. Three species were found, one cestode and two nematodes. Unfortunately the cestodes have been badly injured by preservation in alcohol in such a way that I cannot discover the scolex. Consequently no precise determination of this species was possible. The two nematodes belong to the family Trichuridae, genus *Capillaria*. One, *Capillaria oesophagicola* Soltys, was found in the oesophagus and the other, *C. incrassata* Diesing, in the urinary bladder.

(1) *Capillaria* (=*Eucoleus*) *oesophagicola* Soltys, 1952 (=*C. blarinae* Ogren, 1953)

In a study upon the helminth parasites of the common shrew, *Sorex araneus* L., from the Bialowieza National Park in Poland, Soltys (1952) described a new species of *Capillaria*, found in the oesophagus of this Soricidae and called it *C. oesophagicola*. The description, as follows, given by the Polish helminthologist is very short.*

* From the English summary of Soltys's paper.

"Female, 14–17 mm. in length, broad 0·068 mm. Vulva from the anterior end short of 3·9 mm. Eggs 0·07 × 0·03 mm. The oesophagus ended with two cells. Male, 12 mm. in length. The copulation sheath in the paraterminal part, covered with numerous spines. Lack of spicula. Terminal part of the body slightly split into two parts, behind the split projects a delicate transversely placed crest. *C. oesophagicola* sp.n. are parasites firmly grown into the epithelium of the oesophagus and they are arranged undulately".

Ogren (1953) gave the description of a *Capillaria* parasitic in the oesophagus of the American shrew, *Blarina brevicauda* Say., and called it *Capillaria blarinae*. In general the description of *C. blarinae* was in agreement with that of *C. oesophagicola*, except that Ogren mentioned a spicule.

Soltys (1954) in a new paper on parasites of shrews gave a more detailed description of *C. oesophagicola* and pointed out the existence of a spicule in the male. In a recent paper (Bernard, 1960b) I have provided some details on *C. oesophagicola* found in Continental shrews. Table 1 gives the measurements of *C. oesophagicola* from Poland compared with those of *C. blarinae* Ogren and material collected in the Continental common shrew and British water shrews. It appears from this table that there is no biometric reason to make any distinction between *C. oesophagicola* Soltys and *C. blarinae* Ogren. Moreover, some meristic characters are very similar. In the females, there is no special formation at the vulva, but the fore lip is more protuberant than the hind one; in the uterus the eggs are situated one behind each other. In the males, the body ends with two knob-like formations bearing a "bursal translucent membrane" and the cirrus is similar. Biologically, the two species are also very close; they are found firmly sunk in the oesophageal mucous membrane, in tunnels where the body is undulated. In accordance with all these facts I consider *C. blarinae* Ogren, 1953 as a synonym of *C. oesophagicola* Soltys, 1952, this later name having been the first used for the species.

In his paper, Los Capillariinae, Lopez-Neyra (1947) divided this sub-family of Trichuridae into six genera. According to the Spanish helminthologist, *C. oesophagicola* belongs to the genus *Eucoleus* in which the oesophagus is about one third of the total body length and in which the males have a spicular sheath or cirrus. A description of the material found in British water shrews is given below. (See also Table 1).

TABLE 1

Comparative measurements of *C. oesophagicola* from different origins and *C. blarinae*

	Females				Males			
	C. oesophagicola			*C. blarinae*	*C. oesophagicola*			*C. blarinae*
	Poland	Belgium	England		Poland	Belgium	England	
Total length	14–17 mm.	10–17 mm.	10–11 mm.	10–18 mm.	7–12 mm.	8–10 mm.	9·25 mm.	6–12 mm.
Length of oesophageal region	4·92 mm.	3–4 mm.	3–4 mm.	4·2–6·2 mm.	2·5–4·2 mm.	3–6 mm.	3·0 mm.	2·5–4·2 mm.
Length of the body	3·9–6 mm.	7–14 mm.	7 mm.	6–12 mm.		5–8 mm.	6·25 mm.	3·5–8·0 mm.
Distance between the vulva and the posterior end of the oesophagus		53–200μ	40–60μ					
Length of the vagina		152–433μ	±500μ					
Length of the spicule					400–800μ	300μ	250μ	173–363μ
Length of the cirrus					181μ	150–230μ	420μ	116–128μ
Diameter at :								
—anterior end	13–14μ	11–22μ	15μ		9μ	11–22μ	23μ	
—level of the posterior end of the oesophagus	46–66μ	57–83μ			49–59μ	53–76μ	45·2μ	
—level of the vulva	68–70μ	61–98μ	68–76μ	59–79μ				
—level of the anus	33μ	22–45μ	30μ		30–49μ	22–45μ		
Greatest diameter	82μ	72–121μ	80–83μ	73–96μ	59–98μ	64–91μ	57μ	49–76μ
Eggs : length	60–70μ	57–72μ	60–68μ	54μ				
breadth	26–30μ	26–30μ	22–30μ	24μ				

C. oesophagicola is a typically shaped Capillariid nematode of small size. There is no sharp difference in breadth between the oesophageal region and the anterior extremity of the body itself.

Females : Total length, between 10 and 11 mm. There is a distance of about 7 mm. from the vulva to the posterior extremity and 3 to 4 mm. for the setal part. The diameter is 15μ at the anterior extremity ; it widens progressively until the level of the vulva, where it is 68 to 76μ. The largest diameter is 80 to 83μ and is situated at mid-distance between vulva and anus. This last is subterminal and at its level the breadth is 30μ. The vulva opens near the junction of oesophagus and intestine, sometimes 40 to 100μ behind. There is no special cuticular formation near the vulva but the fore lip is more prominent than the hind one. There is a muscular vagina of 500 to 532μ ; the uterus is 3·5 mm. long. In the latter, about 150 mature eggs are to be seen one behind each other. The length of the ovary is 2·6 to 3·27 mm. The lemon shaped eggs are 60 to 68μ long and 22 to 30μ broad. Their outer surface is marked by irregularly spread punctures.

Male : Total length, 9·25 mm., of which 3 mm. is occupied by the oesophageal part. Anterior extremity of the rostrum 23μ wide, increasing progressively to 45μ at the level of the hind extremity of the oesophagus. Maximum diameter, 57μ, in the third part of the body ; at the level of the anus it is 30·5μ. The anus is terminal. The posterior extremity of the body is divided into two lobes joined by a thin membrane on one side. The hind part of the rectum is a cirrus or copulation sheath internally covered with spines for a length of about 400μ. The spicule is very difficult to observe without dissecting the worm. Sometimes it is possible to extrude artificially the copulation organs *post mortem* by compression in lactophenol. In this manner the cirrus is evaginated and the spicule appears distinctly (Fig. 1). The length of the latter is about 250μ.

The measurements of the worms are in good agreement with those given by other authors for *C. oesophagicola* and *C. blarinae*. We observed, only in the females, that the distance between the vulva and the hind extremity of the oesophagus is very short and in the males, a cirrus twice as long as in the material found in terrestrial shrews (see Table 1).

C. oesophagicola is an intrahistic* parasitic helminth. In general the worms are buried between the mucous membrane and the

* "Intrahistic"=living in the tissue ; a word used by Lopez-Neyra to designate helminths not living in the lumen of an organ.

muscular tissue, the body undulantly stretched along the organ. Sometimes eggs are agglutinated along the body of females.

I found two females and one male in one of the *Neomys fodiens* caught near Oxford. In common shrews on the Continent I sometimes found up to 20 nematodes in one mammal. In this case the greatest number of the parasites are in the lumen of the organ, with their rostrum embedded in the tissue.

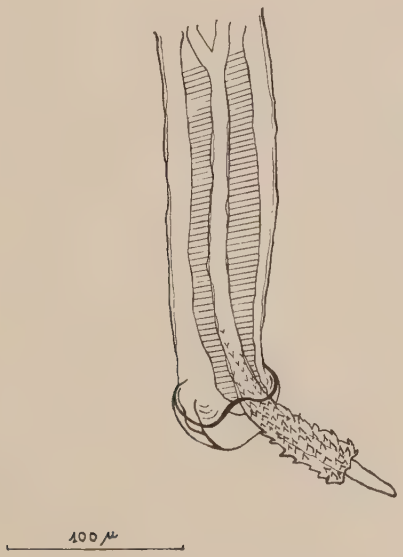


Fig. 1.—*Capillaria oesophagicola* Soltys, 1952. Hind part of the male body, with copulatory organs partly extruded.

At present this species is known only from the red-toothed shrews, *Sorex araneus*, *S. minutus*, *Neomys fodiens* and *Blarina brevicauda*. Although I have dissected a great number of white-toothed shrews of the genus *Crocidura*, I never found this parasite in this host. It seems certain that *C. oesophagicola* is restricted to the members of the sub-family Soricinae.

The geographical range is very wide. The species is known from Poland (Soltys, 1952), Germany (Stammer, 1955), France and Belgium (Bernard, 1960a and b) and from North-America (Ogren, 1953).

The ecological range of the species seems varied. *C. oesophagicola* is found in water shrews as well as in other shrews, living in dry places, in afforested or cultivated areas, along streams and high in the mountains.

(2) *Capillaria incrassata* Diesing, 1851

This species was described by Diesing in 1851 from helminths found in the bladder of a *Crocidura* sp. In 1882, von Linstow found in the same organ of a mole, a male and remains of a female of a Capillariid that he described under the name, *Capillaria capillaris*. von Linstow named as *C. incrassata*, some helminths found in cystic formations of the skin of the testis of *Sorex tetragonus*. All modern helminthologists think that there must be some mistake which gave rise to this last opinion.

Until this time several authors have found *Capillaria* sp. in the bladder of Insectivores and called them *C. incrassata* or *C. capillaris*, in general, without giving an explanation of this choice. In their work upon the helminths of *Crocidura russula*, Joyeux and Baer (1937) gave a new and complete description of *C. incrassata* Diesing, 1851. This description could be applied to the Capillariid found in Belgium in the urinary bladder of the following Insectivores : *Sorex araneus*, *S. minutus*, *Neomys fodiens*, *Crocidura russula* and *C. leucodon*. The helminths discovered in the bladder of water shrews near Oxford are also in agreement with this description. I give here some details upon this material :

Female : Total length, 13 to 13·5 mm., of which the rostrum occupies 8·5 mm. Diameter 13·5μ at the anterior extremity and 91μ at the level of the vulva. Between this and the posterior extremity, the body is almost cylindrical with a maximum breadth of 150μ and 38μ at the level of the anus. The vulva is situated near the junction of the oesophagus and the intestine. In gravid females, the fore lip is very protuberant, making a knob partly covering

the aperture. The vulva is followed by a muscular vagina, 110 to 120μ long. The eggs are lemon shaped, with fusiform patterns on their outer surface. Their measurements are 57μ long and 26 to 30μ broad. About 200 of them are visible, filling the uterus abreast.

Male : The male is normally shorter than the female and the rostrum, between the anterior extremity and the base of the oesophagus, is in general shorter than the second part of the animal. Total length is about 9 to 10 mm., of which the oesophageal region occupies 3 mm. The latter is very thin, increasing in diameter only just before its end where it is about 50μ broad. The visceral part is cylindrical with a diameter becoming rapidly wider after the junction of the oesophagus and intestine, up to 100μ. The spicule, in the specimen from British water shrews, is 1026μ long and 8μ broad. It is enclosed in a sheath which evaginates when the spicule is extruded. In my material the outer part of this sheath is about 100μ long and 16μ broad. The anus is subterminal and the part of the body situated behind it is indistinctly divided into two lobes.

C. incrassata is very common in the urinary bladder of several members of the family Soricidae.

The geographical range of the species covers the same area as that of the host species. At present it is known from France, Belgium (Bernard, 1960a), Germany (Stammer, 1955) and Switzerland (Joyeux and Baer, 1937). In England, Baylis (1928) has observed *C. incrassata* in common shrews near Oxford.

The number of *C. incrassata* that I found in England was a single female in one case and three females and a male together in another case. In Continental water shrews I observed sometimes up to 35 of these helminths, but in general the number lies between 2 or 3 to 10.

One of the water shrews in the urinary bladder of which *C. incrassata* was found was also parasitized by *C. oesophagicola.*

REFERENCES

BAYLIS, H. A., 1928.—"Records of some parasitic worms from British vertebrates". *Ann. Mag. nat. Hist.*, Ser. **10**, 1, 329–343. (W.L. 1050)

BERNARD, J., 1960a.—"Quelques espèces d'helminthes de micromammifères récoltés en France et en Espagne". *Vie et Milieu*, (sous presse). (W.L. 22540a)

————, 1960b.—"A propos de *Capillaria oesophagicola* Soltys, 1952 (=*C. blarinae* Ogren, 1953) parasite de l'oesophage des Soricidae". *Bull. Inst. agron. Gembloux*, **28**, 1–9. (W.L. 4323)

JOYEUX, C. and BAER, J. G., 1937.—"Quelques helminthes nouveaux et peu connus de la musaraigne, *Crocidura russula* Hern.". *Rev. suisse Zool.*, **44**, 27–40. (W.L. 19288)

LOPEZ-NEYRA, R. P., 1947.—"Los Capillariinae". *Mem. R. Acad. Madr.*, **12**, 1–248. (W.L. 13439)

OGREN, R. E., 1953.—"*Capillaria blarinae* (Nem. Trichuridae) from the oesophagus of the short tailed shrew *Blarina brevicauda* Say." *J. Parasit.*, **39**, 135–138. (W.L. 11428)

SOLTYS, A., 1952.—"The helminths of common shrew (*Sorex araneus* L.) of the National Park of Bialowieza (Poland)". *Ann. Univ. M. Curie-Sklodowska*, **6**, Sect. C, 165–209. (W.L. 974a)

————, 1954.—"Helminthofauna of Soricidae in the Bialowieza National Park. *Acta Parasit. Polonica*, **1**, 353–402.

STAMMER, H. J., 1955.—"Die Parasiten deutscher Kleinsäuger". *Verh. dtsch. zool. Ges.*, 362–390. (W.L. 22270)

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, pp. 69–90.

Paramphistomum phillerouxi sp. nov. (Trematoda: Paramphistomatidae) and its Development in *Bulinus forskalii*

By J. A. Dinnik

East African Veterinary Research Organization, Muguga, Kenya

During a study of worm infestations of domestic animals and their control carried out in 1955–1956 in Rhodesia and Nyasaland, Dr. P. L. LeRoux recovered from cattle at Mazabuka in Northern Rhodesia numerous specimens of a conical fluke of the genus *Paramphistomum* Fischoeder. At the same time, specimens of *Bulinus forskalii* (Ehrenberg), emitting paramphistome cercariae, were found in the area where the infested cattle grazed. Encysted cercariae obtained from the snails were sent to the East African Veterinary Research Organization, Muguga, where three calves were fed with 3,166, 1,784, and 1,974 cercariae respectively. All three calves began to pass paramphistome eggs in the faeces 99, 100, and 102 days respectively after they had been given the encysted cercariae. These calves were subsequently used as a source of eggs for hatching miracidia for experimental infection of snails in a study of the larval development of the species.

After observations on development in a snail host had been completed, the calves were killed on the 285th, 479th, and 557th days after infection respectively, when 1,662, 1,841, and 1,931 specimens of one and the same species of *Paramphistomum*, identical to that found in naturally infested cattle at Mazabuka, were recovered from the rumina and, in much smaller numbers, from the reticula of the animals.

It is proposed to name this species, a description and differential diagnosis of which follows, *Paramphistomum phillerouxi* in honour of Philippus L. LeRoux, who first recognized in 1930 the potential harm which conical flukes might cause to the developing animal husbandry of Africa, who has continued to collect material on the distribution and biology of numerous species of these parasites, and who has now found this species and discovered its snail host in Northern Rhodesia. I wish to express here my gratitude to Dr. LeRoux for providing me with the initial material for the study on *P. phillerouxi* and for his constant interest and help in this work.

Paramphistomum phillerouxi nov.sp.

The mature specimens of *P. phillerouxi*, i.e. those which contain eggs in the uterus, vary from 5·0 to 10·0 mm. in length and are conical in shape with the greatest width of the body attained just anterior to the acetabulum where it is from 1·7 to 3·1 mm. if measured dorso-ventrally (Fig. 1). The body surface is marked by well defined transverse wrinklings. The genital pore is clearly seen lying encircled by a narrow swelling and it is situated on the mid-line of the ventral surface about one-fifth to one-quarter of the length of the body from the anterior end. A number of minute papillae are scattered in the area around the genital pore and in the area between it and the anterior end of the worm.

Table 1

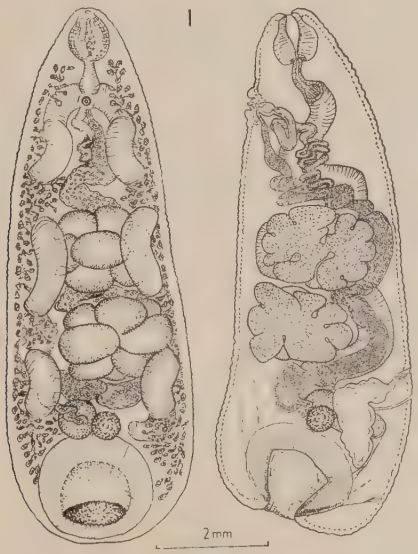
The number of units in the circular muscle series of the acetabulum of *Paramphistomum phillerouxi* sp.nov.

Body length	Diameter of the acetabulum	Number of units in the various circular series				
		d.e.1	d.e.2	d.i.	v.i.	v.e.
5·0 mm.	1·3 mm.	26	17	46	44	19
6·3 mm.	2·0 mm.	21	22	49	48	11
7·2 mm.	2·0 mm.	19	30	47	57	18
7·0 mm.	2·1 mm.	20	28	46	49	18
7·6 mm.	2·2 mm.	15	30	51	54	19
6·2 mm.	2·2 mm.	19	30	48	43	22
7·9 mm.	2·3 mm.	27	23	53	53	22
7·3 mm.	2·3 mm.	17	27	44	54	16
8·7 mm.	2·4 mm.	22	30	48	44	20
7·7 mm.	2·5 mm.	23	28	50	55	19

The acetabulum is subterminal and its diameter is one-fourth to one-third of the body length. The external diameter of the acetabulum measured dorso-ventrally varies according to the size of the worm from 1·3 to 2·5 mm., being two to three times greater than the length of the pharynx.

The acetabulum is of the *Paramphistomum* type with the dorsal exterior circular layer of its musculature clearly divided into two parts. The number of units in various circular muscle series, as they are seen in the sagittal section of the acetabulum is shown in Table 1.

The pharynx is 0·7–1·0 mm. long, being one-seventh to one-ninth of the body length. As seen in the sagittal sections of the pharynx, its internal circular layer consists of well defined units arranged in a single row extending from the anterior part to the basal part of the pharynx. These units are smallest in the anterior part, in the middle part there are from 7 to 11 units in 0·1 mm. length, and the size of the units increases gradually towards the posterior end of the pharynx. In the basal part the units are expanded in the radial



Paramphistomum phillerouxi n.sp.
Fig. 1.—Ventral view and a thick sagittal section of 285 days old type specimen.

direction and are more tightly packed together, forming a kind of a weak posterior sphincter. The interior longitudinal layer occupies about one-sixth of the thickness of the pharyngeal wall and comprises fine muscle fibres running antero-posteriorly along the inner border of the wall. The layer has an indistinct exterior limit. The middle circular layer is weakly developed but well seen as groups of muscle units arranged in a row running along the external limit of the interior longitudinal layer. In the basal part of the pharynx the series of the middle circular layer turn outwards and join with

that of the exterior circular layer. Both series also merge together in the anterior part of the pharynx and here strong muscle bundles occur ; further forward very small muscle units continue the middle circular layer to the anterior end of the pharynx. The radial layer consists of numerous bundles of many tightly packed fibres running across the pharyngeal wall. The bundles diverge into separate fibres at both their ends, before passing between the external circular units and in the field of the interior longitudinal layer. The external circular layer is clearly seen as groups of loosely packed muscle units arranged along the outside border of the pharynx at the distance of about one-sixth to one-eighth of its wall width. This layer contains, amongst other things, somewhat oblique muscle elements. The external longitudinal layer is very narrow, forming only about 1/16 to 1/20 of the thickness of the pharyngeal wall. The basal circular layer is well developed, consisting of 11 to 22 muscle units which lie in two rows in the internal and in one row in the external part of the series. The posterior sphincter is indistinct although the stronger units of the interior circular layer, together with muscle units lying between them, and the units of the middle circular layer all form the muscle complex in the basal part of the pharynx which may be regarded as a weakly developed posterior sphincter. Anterior and lip sphincters are absent.

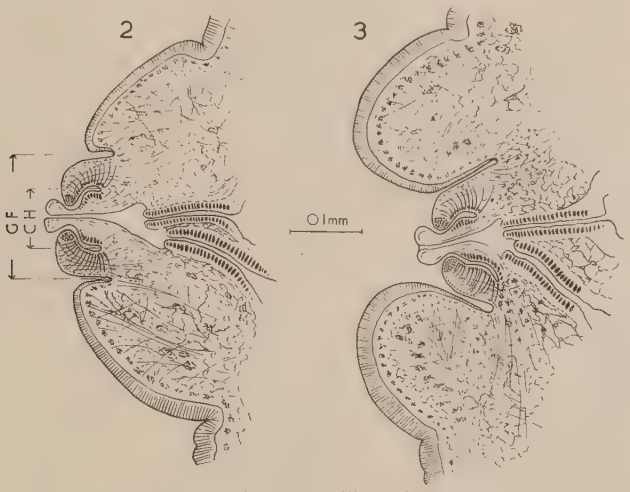
The oesophagus is shorter than the pharynx, being 8/10 to 6/10 of the length of the latter. The wall of the oesophagus is of uniform thickness throughout its length and its muscular part is composed of an exterior layer of longitudinal fibres and an internal layer of circular fibres. The muscle units of the internal layer are arranged in two irregular rows.

The two gut caeca running posteriorly make several identical bends on either side of the body and reach the level of the acetabulum. The terminal parts of the caeca are turned dorsally with the blind ends pointed inwards. The blind ends are short and lie in the lateral areas of the body not extending far across its dorsal side.

The excretory bladder is flask-shaped and has a well-developed excretory canal. The excretory pore opens in the third quarter of the body length at about the level of the second testis or its posterior border at a distance of 2·5–3·1 mm. from the posterior end of the body.

The testes are situated one behind the other in the midthird of the body. Both are approximately of the same size, 0·6–1·3 mm. long and 0·8–2·5 mm. wide, and are deeply lobed having from 10 to 15 large lobes each.

The ovary lies between the second testis and the inner border of the acetabulum in the middle of the body and is ventral to the excretory bladder. Mehlis' gland lies close to the ovary on its right or left side. Laurer's canal runs from the ovary and Mehlis' gland to the dorsal side of the body crossing the excretory bladder in its middle part. The opening of Laurer's canal is situated 0·4–0·7 mm. posterior to the excretory pore.



Paramphistomum phillerouxi n.sp.

Fig. 2.—Genital atrium in its protruded stage in a specimen of 8·0 mm. long.
Fig. 3.—Genital atrium in its retracted stage in a specimen of 7·6 mm. long.
GF—diameter of the circular fold of the genital atrium; **CH**—diameter of the genital chamber.

Clusters of vitelline glands extend from the pharynx to the level of the middle or posterior part of the acetabulum on both sides of the body.

The genital atrium lies at or slightly posterior to the level of the gut bifurcation. It is of the *Microbothrium* type. The circular fold of the genital atrium is well developed and clearly outlined. The radial musculature of evenly curved bundles of fibres running from the inner to the outer surface of the fold and the strong and well delimited genital sphincter are well seen in the genital atrium. The genital papilla has a strong sphincter consisting of circular muscle units arranged in a row in the outer side of its basal part. Fine fibres of the retractor muscle are seen running through the basal part to the distal end of the papilla (Figs. 2 and 3).

In the mature specimens (7–10 mm. long) the circular fold of the genital atrium is 0·147–0·193 mm. in diameter, the muscle of the genital sphincter is 0·016–0·023 mm. thick, and the diameter of the inner chamber, with the papilla inside, is 0·075–0·105 mm. The diameter of the circular fold of the genital atrium is 1/4–1/6 and the diameter of its inner chamber is 1/8–1/10 of the pharynx length.

The pars prostatica is clearly delimited from the genital atrium by a narrow ductus ejaculatorius and from the pars musculosa by a constriction with a narrow canal through it. The pars prostatica is 0·17–0·28 mm. long and its length is only slightly greater or is equal to its width.

The pars musculosa is 0·14–0·24 mm. wide and its muscular wall is 0·030–0·045 mm. thick in the part close to the pars prostatica. The pars musculosa is of moderate length making only two or three convolutions on its way from the vesicula seminalis to the pars prostatica.

The vesicula seminalis is seen as an irregular tangle of thin-walled coils lying anterior to the testes and slightly dorsal to the midline of the body (Fig. 4).

The uterus runs from Mehlis' gland first towards the ventral part of the body then it turns dorsally and passes between the ovary and the posterior testis to the dorsal part of the body. After that the uterus turns to run forwards and winds behind the testes to the level of the anterior border of the first testis where the uterus turns ventrally again and passes over the testis and between the vasa deferentia to the ventral surface of the worm. The muscular metraterm links the uterus with the genital atrium by opening into the cavity in the base of the genital papilla just below the ductus ejaculatorius.

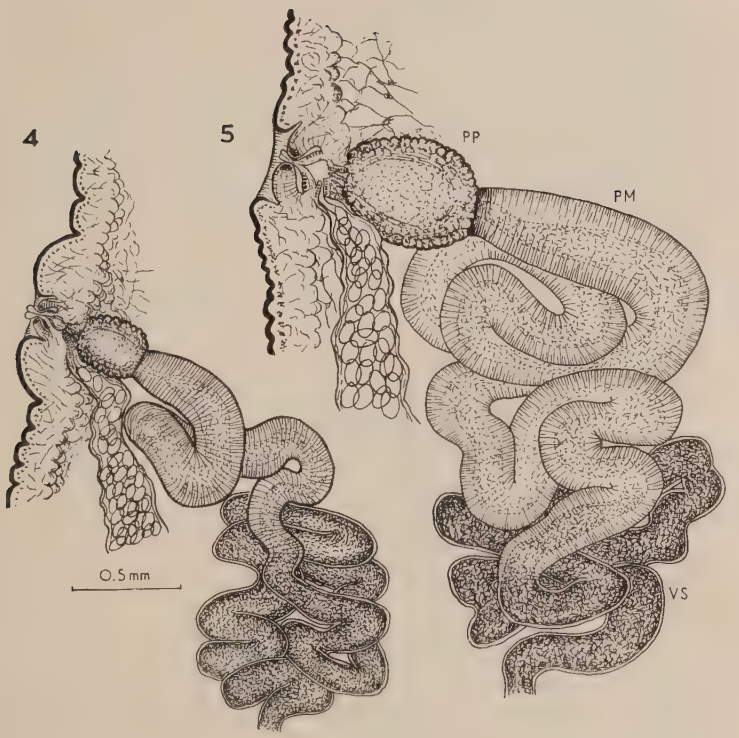
The eggs are 0·130–0·157 mm. long and 0·067–0·085 mm. wide.

Type : The specimens which were recovered from the rumen of *Bos indicus* to which metacercariae obtained from *Bulinus forskalii* (Ehrenberg) had been fed.

Type locality : Mazabuka, Northern Rhodesia, Africa.

The type and co-type specimens of *P. phillerouxi* are in the collections of British Museum (Natural History), London.

Other localities : Chinsali, Monza and Lusaka Districts of Northern Rhodesia ; Inyanga in Southern Rhodesia ; Mwanza and Musoma District of the Lake Province in Tanganyika ; Kamba District in Kenya ; and Mauritius Island.



Paramphistomum phillerouxi n.sp.

Fig. 4.—Genital atrium, pars prostatica, pars musculosa, and vesicula seminalis in 10 mm. long and 285 days old specimen.

Paramphistomum microbothrium Fisch.

Fig. 5.—Genital atrium, pars prostatica (**PP**), pars musculosa (**PM**), and vesicula seminalis (**VS**) in a specimen of 11 mm. long from the rumen of *Bos indicus*, Kitale, Kenya. (Both drawn to same scale.)

Relationships

The new species, *Paramphistomum phillerouxi*, belongs to a group of the following species of the genus : *P. microbothrium* Fischoeder 1901, *P. clavula* Nasmark, 1937, *P. microbothrioides* Prince et McIntosh, 1944, and *P. sukari* Dinnik, 1954. The common characteristics of this group are that all have a well developed fold of the genital atrium with a genital sphincter, and the genital papilla is provided with a strong sphincter papillae. In all these species the genital atrium is neither situated at the bottom of a deep and large ventral atrium (as in *P. bothriophorum* [M. Braun]) nor is it exposed at the top of a long ventral papilla (as in the species of the genus *Calicophoron* Nasmark).

P. clavula Nasmark is distinguished among these species by its large genital atrium with an enormously strong genital sphincter which is about 0·12 mm. across, i.e. it is about six times larger than that of *P. phillerouxi*.

The new species differ from *P. microbothrioides*, the description of which is very brief, by the structure of the acetabulum which in *P. phillerouxi* has the dorsal part of the exterior circular musculature divided into two clearly separated parts of which the second part (d.e.2 circular) consists of 21 to 30 evenly spaced small units.

P. phillerouxi differs from *P. sukari* by the structure of the acetabulum which in *P. sukari* has not d.e.2 circular series, by the more lobed testes, and the position of the terminal parts of the caeca which in *P. sukari* are turned ventrally.

P. phillerouxi closely resembles *P. microbothrium* and could easily be mistaken for the latter species if only the diagnostic criteria given by Nasmark were used. The structure of the acetabulum, the pharynx and the genital atrium afford good possibilities of differentiating some of the *Paramphistomum* species as shown by Nasmark (1957) but in this case the descriptions of these anatomical structures in *P. microbothrium* were found insufficient for the differentiation of these two species of the genus. The differences between the two species have been established by comparing the morphology of the new species with that of *P. microbothrium* both from descriptions in the literature and also by a study of several specimens of the latter species collected by Dr. P. LeRoux from cattle and sheep at the Cairo abattoir in Egypt.

The first brief description of *P. microbothrium* was given by Fischoeder in 1901 and was repeated in his dissertation published in 1902. A more detailed description of the species appeared in Fischoeder's monograph on the paramphistomes of mammals, 1903.

The material which Fischoeder used for the description of *P. microbothrium* comprised 14 paramphistomes of 8 to 11 mm. long recovered from *Antilope dorcas*. This antelope, the name of which is a synonym of *Gazella dorcas* (L.), occurs in North Africa from Morocco to Ethiopia and, presumably, the animal from which the paramphistomes were obtained originated from this area.

Specimens of *P. microbothrium*, from the rumen of *Bos taurus* and *Bos bubalis* slaughtered at Cairo, were found by Nasmark (1937) in the collections of the late Prof. A. Looss and also in those of the Swedish Sudan Expedition, 1900–1901. Nasmark compared this new material with the original specimens of the species and confirmed the correctness of Fischoeder's description. In addition Nasmark gave a detailed description of the structures of the pharynx, the acetabulum and the genital atrium of *P. microbothrium*.

An examination of several specimens of *P. microbothrium* collected by LeRoux from cattle and sheep in Cairo has shown that their morphology, including the structures of the pharynx, the acetabulum and the genital atrium, conforms exactly to the descriptions and illustrations of Fischoeder (1903) and Nasmark (1937). These specimens of *P. microbothrium* from Egypt were compared with mature specimens of the paramphistome from Kenya which were obtained for the experimental study on their life-history (Dinnik and Dinnik, 1954) and which were referred to as *P. microbothrium*. The specimens from Kenya and Egypt were exactly alike, both in their general morphology and in the detailed structure of the pharynx, acetabulum and genital atrium. A great number of sectioned specimens of *P. microbothrium* from cattle, sheep and goats from Kenya, Uganda, Tanganyika, Northern Rhodesia and South Africa were examined and were found to be identical to the specimens from Egypt. This study of the morphology of *P. microbothrium* from Africa and the comparison of it with that of *P. phillerouxi* has made it possible to differentiate these two species by the following most characteristic features.

1. The genital atrium of *P. phillerouxi* is only half the size of that of *P. microbothrium*. In Fischoeder's description of the species the diameter of the atrium is 0·25–0·30 mm. and the muscle of its sphincter is 0·03–0·04 mm. thick. Nasmark (1937, Fig. 69) shows, in an illustration of the genital atrium of *P. microbothrium*, that the circular fold of the genital atrium is 0·292 mm. in diameter, that the genital chamber is 0·209 mm. in diameter, and that the genital sphincter muscle is about 0·05 mm. thick. The measurements of the genital atrium of two specimens of *P. microbothrium* 6·6 mm. and 7·3 mm. long recovered from cattle in Cairo were as

follows : the diameter of the genital fold, 0·287 mm. and 0·294 mm.; the genital chamber, 0·131 mm. and 0·150 mm. in diameter and the muscle of the genital sphincter, 0·033 mm. and 0·049 mm. thick. In specimens of *P. microbothrium*, 7 to 11 mm. long, collected in Africa South of the Sahara, the diameter of the fold of the genital atrium is 0·237–0·408 mm., the genital chamber 0·150–0·220 mm. in diameter, and the muscle of the genital sphincter is 0·033 mm.–0·059 mm. thick.

In specimens of *P. phillerouxi*, 7–10 mm. long, the circular fold of the genital atrium is 0·147–0·193 mm. in diameter, the diameter of the genital chamber is 0·075–0·105 mm., and the genital sphincter is 0·016–0·023 mm. thick.

In relation to the pharynx the diameter of the fold of the genital atrium in *P. microbothrium* is about 4/10 and in *P. phillerouxi* is about 2/10 of the pharynx length.

2. The pars musculosa of *P. microbothrium*, according to Fischoeder, is very much coiled and as seen in the illustrations (Fischoeder, 1903, Figs. 21 and 22) it has many convolutions on its way from the vesicula seminalis to the pars prostatica. A long pars musculosa with many convolutions was found in all specimens of *P. microbothrium* from Egypt and the other parts of Africa. Its width was found to vary from 0·26 to 0·39 mm. (Fig. 5). In *P. phillerouxi* the pars musculosa is shorter, having only two or three convolutions on its way to the pars prostatica, and it is narrower than that of *P. microbothrium*, being only from 0·14–0·24 mm. in width.

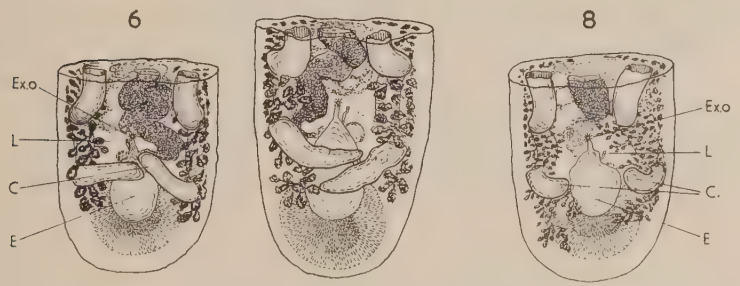
In both species the length of the pars prostatica is only slightly greater than its width or equal to it, but in *P. microbothrium* it is 0·40–0·53 mm. long whereas in *P. phillerouxi* the pars prostatica is only 0·17–0·28 mm. long.

3. In the original description of *P. microbothrium* it is stated that the posterior parts of the gut caeca turn dorsally and inwards to lie 1·0 to 1·5 mm. anterior to the acetabulum. In all specimens of *P. microbothrium* from Egypt and Africa South of the Sahara it was found that the terminal parts of the gut caeca were invariably turned dorsally and inwards so that their blind ends were clearly seen on the dorsal side of the body where they were situated between the excretory bladder and the dorsal wall of the body (Figs. 6 and 7). The blind ends overlapped each other or, rarely, reached each other in the middle region of the dorsal side of the body. In median sagittal sections of the worms both ends of the gut caeca were seen

cut across and situated one posterior to the other between the excretory bladder and the dorsal wall of the body.

In *P. phillerouxi* the terminal parts of the caeca are also turned dorsally and slightly inwards but their blind ends were never seen to extend across the dorsal side of the body to its middle region. In the specimens viewed from the dorsal side the blind ends were seen lying on both sides of the acetabulum and in median sagittal sections they could not be seen (Fig. 8).

7



Paramphistomum microbothrium Fisch.

Dorsal view of specimens showing the position of the terminal parts of the caeca. Fig. 6.—*P. microbothrium* from a cow slaughtered at Cairo, Egypt. **Ex.o**—excretory opening; **L**—opening of Laurer's canal; **C**—terminal part of caecum; and **E**—excretory bladder. Fig. 7.—*P. microbothrium* from a cow, Kenya.

Paramphistomum phillerouxi n.sp.

Fig. 8.—Dorsal view showing the position of the terminal parts of the caeca in the co-type specimen.

4. In specimens of *P. microbothrium* Laurer's canal crossed the excretory bladder in its anterior part running from the ovary and Mehlis's gland in an antero-dorsal direction, its pore opening 0·25–0·50 mm. posterior to the excretory pore. In specimens of *P. phillerouxi* Laurer's canal ran almost straight to the dorsal side where it opens 0·4–0·7 mm. posterior to the excretory pore.

5. The eggs of *P. microbothrium*, as measured in the specimens from Egypt, were 0·153–0·186 mm. long and were 0·150–0·202 mm. long in the specimens from Kenya. Fischoeder gave the size of *P. microbothrium* eggs as 0·145–0·150 mm. and Nasmark as 0·156 mm. long. The eggs of *P. phillerouxi* are 0·130–0·157 mm. long.

Nasmark (1937) stated that the pharynx of *P. microbothrium* is of the *Paramphistomum* type which, amongst other features, is characterized by the absence of a middle circular layer. An examination of many good sections of *P. microbothrium* from Egypt and Kenya has revealed the presence of small groups of muscle fibres scattered along the external limit of the interior longitudinal layer of the pharynx. The row of these groups can be regarded as a weakly developed middle circular layer in the pharynx of *P. microbothrium*. Its units are inconspicuous in the middle part of the pharynx but they are more strongly developed and easily seen in its anterior part where they merge with the units of the exterior circular layer and continue as one layer towards the anterior end of the pharynx.

In the pharynx of *P. phillerouxi* the middle circular layer is well developed and easily seen, and therefore, it cannot be referred to the *Paramphistomum* type. However, this difference between the two species is not sufficiently well defined to be used to distinguish the species.

Snail Hosts of *Paramphistomum phillerouxi*

The type specimens of *P. phillerouxi* were reared in a calf from encysted cercariae of the species emitted by naturally infected specimens of *Bulinus forskalii* (Ehrenberg), collected in a paddock of the Central Research Station at Mazabuka in Northern Rhodesia in March 1956. These snails had survived for at least eight months of the dry season, during which time the paddock was waterless. However, soon after the first heavy rainfall of the rainy season many snails of various sizes were seen to be moving in the pools which formed in the shallow depressions of the paddock, and within 48 hours of the rainfall they started to emit paramphistome cercariae (LeRoux, 1957).

Bulinus forskalii emitting paramphistome cercariae was found at Mwanza, Tanganyika in June, 1957, and 857 specimens of *P. phillerouxi* were isolated from a calf killed 348 days after it had been given 1,435 encysted cercariae from these snails.

Experimentally many hundreds of specimens of *B. forskalii* were successfully infected with miracidia of *P. phillerouxi*. The snails used in the experiments were laboratory-reared specimens, progeny of wild *B. forskalii* collected at Mwanza, Tanganyika. Miracidia used for infection of these snails were hatched from eggs obtained from the calves infected with encysted *P. phillerouxi* cercariae shed by specimens of *B. forskalii* collected at Mazabuka, Northern

Rhodesia. The miracidia were readily attracted to the snails, penetrated into the latter within the short time of two to four minutes, and the snails were highly susceptible to the infection. About 70% of the snails which had been each exposed to a single miracidium, and nearly all of the snails which had been placed in water containing numerous swimming miracidia, later became infested with larval stages of *P. phillerouxi*. But in cases of heavy infestation as the result of exposure to numerous miracidia, the infested snails died either before or shortly after they began to emit cercariae.

The finding of *B. forskalii* infected with larval stages of *P. phillerouxi* under natural conditions in Northern Rhodesia and Tanganyika, and the ease with which specimens of this snail species became infected with the paramphistome when exposed to its miracidia under laboratory conditions, both showed that *B. forskalii* is a natural snail host of *P. phillerouxi* in East and Central Africa.

Another snail species which may be a natural intermediate host of *P. phillerouxi* in Africa is *Bulinus senegalensis* (Müller), which is very similar to *B. forskalii* in many respects and is a common snail in West Africa. In four trials a total of 174 laboratory-bred snails, progeny of wild *B. senegalensis* from Gambia, were exposed to *P. phillerouxi* miracidia. The snails became infested with rediae and eventually began to shed cercariae. No differences were noted in larval development of *P. phillerouxi* in *B. senegalensis* and *B. forskalii* respectively ; development proceeded normally in both snail species, and the numbers of rediae and cercariae produced per snail were similar.

A third snail species which could be an intermediate host of *P. phillerouxi* in Africa is *Physopsis globosus* (Morelet). Specimens of this species collected at Mazabuka and Lusaka in N. Rhodesia were found to emit pigmented cercariae of the amphistome type. A number of these cercariae after encystment were fed to a sheep and a calf and young specimens of *Paramphistomum* sp. with a few eggs in the uterus, and of *Carmyerius* sp., were recovered from both animals 105 days later. The *Paramphistomum* specimens could not be distinguished from *P. phillerouxi* specimens of the same age and size, but on the other hand they could not, at this young stage, be identified with certainty as the latter species.

Outside of the African continent, specimens of *Bulinus cernicus* (Morelet) emitting two kinds of pigmented cercariae of the amphistome type were collected by Dr. LeRoux in Mauritius Island in August 1954. When the encysted cercariae were fed to a calf, they developed into *P. phillerouxi* and *Stephanopharynx compactum.*

B. cernicus of Mauritius probably is a synonym of *B. forskalii* of Africa, or closely related to the latter species.

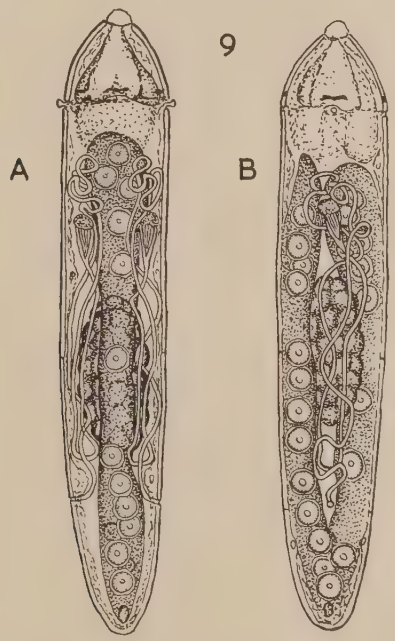
Attempts were made to infect *Bulinus tropicus alluaudi* (Dautzenberg) and *Lymnaea natalensis caillaudi* (Bourguignat) from Kenya with miracidia of *P. phillerouxi*. *B. tropicus* with its subspecies is a natural intermediate host of *P. microbothrium* Fischoeder in East, Central and South Africa. In several attempts to infect them, a total of 200 young laboratory specimens of *B. tropicus alluaudi* were exposed to *P. phillerouxi* miracidia, but none became infected. Attempts to infect 172 specimens of laboratory-bred *Lymnaea natalensis caillaudi* by exposure to *P. phillerouxi* miracidia were also unsuccessful. At the laboratory of the London School of Hygiene and Tropical Medicine, Dr. LeRoux (per. comm.) exposed specimens of *Bulinus truncatus* from Egypt, *Bulinus tropicus* and *B. forskalii* from Rhodesia, *B. senegalensis* from Gambia, and *Physopsis africanus* to miracidiae of *P. phillerouxi*. Only the species *B. forskalii* and *B. senegalensis* proved susceptible and yielded later many cercariae.

Development of *P. phillerouxi* in a Snail Host

The eggs of *P. phillerouxi* are passed in the faeces of an infected animal at an early stage in their embyronic development, when the embryo consists of a single cell, or of two or three cells into which it has divided. The embryo is situated in the centre of the egg shell and is surrounded by yolk cells filled with granules. Miracidia developed and hatched from the egg shell in 15 days when kept in water at 26°C.

The miracidium in a relaxed state varies from 0·20 to 0·23 mm. in length. Its body is covered with 20 ciliated epithelial cells arranged in four transverse rows of 6, 8, 4 and 2 cells each respectively. The excretory system consists of two posteriorly-directed flame cells from each of which a long duct leads to the excretory pore situated near the end of the miracidium body. Each of these ducts first runs posteriorly almost to the level of the excretory pore, where it turns back to return along the lateral side of the body to the flame cell and to extend anteriorly to it for a short distance. Here, near the flame cells, the duct makes a few small loops, turns posterior again and, after passing the flame cell, runs with a slightly winding course to the corresponding excretory pore. The excretory pores open to the outside on the lateral sides of the posterior end of the miracidium at the junction of the 3rd and 4th rows of its epithelial cells (Fig. 9).

The germinal material of the miracidium consists of 19 to 28 cells of irregular shape, packed together in a compact elongated mass which lies in the body cavity. Each germinal cell has a large round nucleus surrounded by a thin layer of protoplasm. In a few miracidia the germinal cells were seen to be in a mitosis stage, but it was not clear whether this represented multiplication of the germinal cells or division of a germinal cell into the first two cells of the embryo.



Paramphistomum phillerouxi n.sp.

Fig. 9.—Miracidium, showing the excretory system, central group of germinal cells, and two granulated masses with round cells in them. **A**—dorsoventral aspect. **B**—lateral aspect.

In the miracidium two granulated masses of protoplasm extend from the posterior end along the dorsal and ventral sides of the body cavity. Large round cells were seen passively floating in these protoplasmic bodies from one end to the other when the miracidium contracted its body. These floating cells, which vary in number

from 18 to 22 in a miracidium, resemble the germinal cells in structure; like the latter, each of the former cells has a large round nucleus covered with a thin layer of protoplasm. No other cells in the miracidium body with the exception of the germinal cells in the central group and the floating cells in the protoplasmic masses show such resemblances in structure or in staining by neutral red. Miracidia of all the species of the family Paramphistomatidae whose structure was studied, namely: *P. microbothrium* Fischoeder, *P. sukari* Dinnik, *Calicophoron raja* Nasmark, *Cotylophoron jacksoni* Nasmark, *Coenocotyle scoliocoelium* Fischoeder, *Carmyerius exoporus* Maplestone, and *C. mancupatus* Fischoeder, were found to possess germinal-like cells floating in two protoplasmic bodies extending from the posterior to the anterior part of the body. The significance of the floating cells in the miracidia remained obscure.

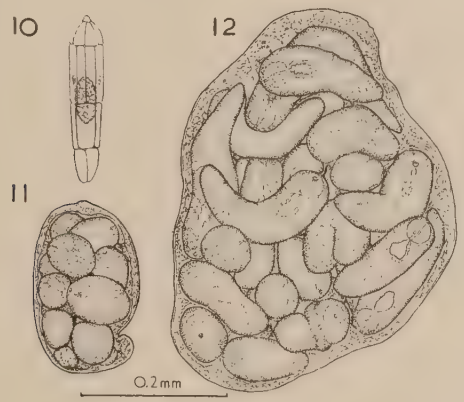
After they had penetrated into a snail, the miracidia or the sporocysts which developed from them were found to be localized in various parts of the snail body; two sporocysts were even seen developing in the tentacles of the snail. However, normal development in which a sporocyst gives rise to rediae which can migrate successfully from the place of their origin to continue their life in the liver took place in the mantle, the tissue of the respiratory cavity, the tissue surrounding the intestine, and in the liver itself.

To follow the course of development of the sporocyst and rediae produced by it, snails were dissected within 10 to 39 days after each had been exposed to a single miracidium. Sporocysts ten days old were found to be only slightly longer, 0·23 to 0·29 mm. in length, but three to four times as wide, 0·14 to 0·17 mm., as the miracidium (Figs. 10 and 11). By that time the germinal cells of the miracidium had developed into embryo balls which entirely filled the body cavity of the sporocyst. The redial structures could already be recognized in only a few of these embryos. The sporocysts recovered from snails dissected 12 and 13 days after exposure to one miracidium each contained from 20 to 31 embryos each, at various stages of development, from a small embryo ball to a clearly-recognized young redia. The number of young rediae seen at that stage in a sporocyst varied from one to eight. By the 15th day of development in the snails the sporocysts had grown up to 0·56 mm. long and 0·45 mm. wide, and each contained from 16 to 23 young rediae and several embryo balls (Fig. 12). The total number of embryos varied from 23 to 33 per sporocyst.

Young rediae moving freely in the snail tissue outside of the sporocyst were first noted in a snail dissected 12 days after its exposure to a miracidium. Seven young rediae were still present

in the ruptured body of the parent sporocyst, while 21 others which had developed in this sporocyst were recovered from the tissue of the snail dissected 15 days after its exposure to a miracidium.

The sporocyst of *P. phillerouxi* has no birth pore and after the young rediae have developed in it they must rupture the wall of the parent in order to escape and to continue their development by feeding on the snail tissue. Once this escape process was observed under the microscope; in a sporocyst containing many young rediae one rediae 0·41 mm. long began to move vigorously and soon succeeded in making a small hole in the wall of the parent sporocyst through which it quickly escaped head first. After the young redia had passed through, the hole closed so that the damage to the wall could not be seen.



Paramphistomum phillerouxi n.sp.

Fig. 10.—Miracidium, showing the arrangement of the epithelial cells and the group of germinal cells. Fig. 11.—10 days old sporocyst. Fig. 12.—15 days old sporocyst with young rediae inside.

The number of rediae recovered from snails each exposed to a single miracidium varied from 18 to 28 per snail. It appeared that the sporocyst of *P. phillerouxi* produced slightly fewer first-generation rediae than the number of germinal cells in the miracidium. It seems also that some of the embryo balls in the sporocysts failed to develop into young rediae even under favourable conditions.

At the time of their escape from the sporocysts, from the 12th day onwards, the young rediae were from 0·20 to 0·43 mm. long and contained germinal cells and a few embryo balls in the body

cavity. Within 3 to 5 days of their free development in the snail tissue they grew to 0·5 to 0·8 mm. in length and the embryo balls in them increased in size and number.

In a snail dissected 15 days after exposure to one miracidium 28 rediae were found; 5 of these, 0·50 to 0·77 mm. long, contained large embyos in the anterior part of the body cavity. Each of these rediae was dissected and examined separately. Two of them were found to contain developing daughter rediae; one had a young daughter redia (with a body 0·17 mm. long and 0·09 mm. wide) and 21 embryo balls, and the other had 2 redial embryos and 19 embryo balls. The three remaining rediae contained only unrecognizable embryos in the form of balls.

From a snail dissected on the 18th day 26 rediae were recovered. Of these, seven of the larger, measuring from 0·57 to 0·81 mm. in length, were dissected in order to ascertain their contents; they were found to contain the following numbers of developing daughter rediae, recognizable redial and cercarial embryos, and embryo balls.

(a) one daughter redia, three cercarial embryos, and 18 embryo balls; (b) one daughter redia, five cercarial embryos, and 18 embryo balls; (c) one redial embryo, five cercarial embryos, and 23 embryo balls; (d) one redial embryo, four cercarial embryos, and 21 embryo balls (Fig. 13); (e) four cercarial embryos and 14 embryo balls; (f) four cercarial embryos and 16 embryo balls; (g) three cercarial embryos and 20 embryo balls.

All the other rediae from the snail were immature; i.e., they did not contain either redial or cercarial embryos recognizable in the body cavities, but only embryo balls. Thirteen of them, from 0·33 to 0·54 mm. long, were young rediae of the first generation, and six, from 0·19 to 0·24 mm. long, were young rediae of the second generation.

Another snail from the same series was dissected on the 21st day, when 10 mature rediae and 11 young immature rediae of the first generation and 4 young rediae of the second generation were found. Five mature rediae were dissected; their sizes and contents were as follows :—

(a) 0·72 mm. long; contained five cercarial embryos and 25 embryo balls; (b) 0·73 mm. long; contained one daughter redia, three cercarial embryos, and 18 embryo balls; (c) 0·75 mm. long; contained five cercarial embryos and 18 embryo balls; (d) 0·87 mm. long; contained five cercarial embryos and 19

embryo balls; (e) 0·91 mm. long; contained one daughter redia, seven cercarial embryos and 20 embryo balls.

Three young cercariae developing freely in the tissue were seen in the dissected snail.

On the 33rd day of infection 61 rediae and 788 free-developing cercariae were recovered from the snail. Of these rediae 29 were mature ; they varied from 0·54 to 1·01 mm. in length and contained from 1 to 4 cercarial embryos and from 8 to 18 embryo balls in their body cavities. No redia with daughter rediae was seen among them.

The last snail of this series, in which each snail had been exposed to a single miracidium, was dissected on the 39th day. The snail was found to contain 58 rediae ranging from new-born rediae 0·19 mm. in length to mature rediae 0·52 to 0·88 mm. in length ; it was impossible to distinguish between the generations among them. Only one mature redia, 0·70 mm. long, was found to contain a daughter redia with 3 cercarial embryos and 6 embryo balls. The remaining mature rediae contained from one to five cercarial embryos and embryo balls. Numerous cercariae in various stages of development, from newly-born with small eyespots to wholly pigmented cercariae, were present in the snail, but none was mature and able to encyst.

The morphology of the first-generation redia of *P. phillerouxi* is similar to that of *P. microbothrium* Fischoeder and *P. sukari* Dinnik ; they do not have a collar and body appendages, and the only difference noted is in the dimensions of the body parts.

Many snails in several series were exposed to 3–5 miracidia each and were dissected within the period when rediae of the first generation were expected to reach maturity and redial or cercarial embryos appeared in their body cavities. These mature rediae varied from 0·52 to 1·17 mm. in length and from 0·17 to 0·23 mm. in width. The muscular pharynx was 0·043 to 0·046 mm. wide and 0·033 to 0·043 mm. long, and the sac-like gut extended from the pharynx to the level of the birth pore or ended at a point slightly anterior to it. As in all the known Paramphistomatidae, the redia of *P. phillerouxi* has three pairs of flame cells. Each mature redia examined contained embryo cells at the posterior end, from which embryo balls originated. The size of the balls gradually increased towards the anterior part of the body cavity, where they became recognizable as either redial or cercarial embryos. In some parent rediae the first one or two embryos were redial embryos or even daughter rediae, while the several following ones were cercarial embryos.

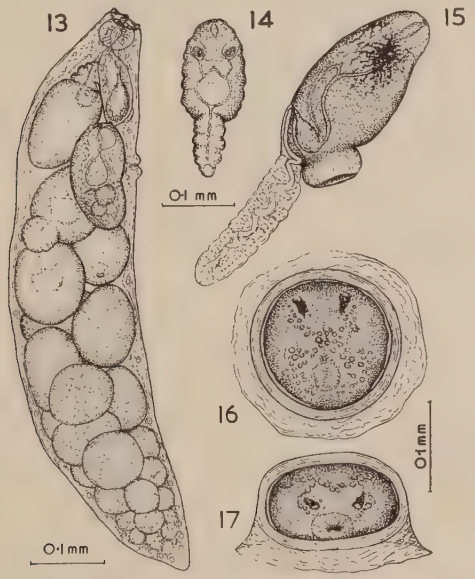
In other rediae all the first embryos were cercarial. It appeared that not all of the first-generation rediae commenced with the production of daughter rediae, as is the case with *P. microbothrium* and *P. sukari* (Dinnik and Dinnik, 1954 and 1957). In the larval development of *P. phillerouxi* some rediae first began to produce cercariae. However, observations indicated that these rediae were later able to produce a few daughter rediae as well as the numerous cercarial embryos.

It was difficult to keep infected *Bulinus forskalii* alive under laboratory conditions for more than a month, and only a few infected snails survived for much longer than 44 days after exposure to miracidia, when they usually started to emit cercariae. Mature rediae containing redial embryos were observed in all snails dissected after that time. For example, from a snail dissected on the 46th day numerous rediae were recovered, among which were found four old rediae of the first generation. One of the old rediae contained a daughter redia, two developing cercariae, and one embryo ball. Of the rediae of subsequent generations, 12 were dissected and redial embryos were seen in 3 of them. The last infected snail was examined 63 days after exposure to miracidia; it contained numerous rediae among which there were newly born young rediae, rediae producing daughter rediae and cercariae, and 3 old first-generation rediae one of which had one daughter redia and three embryo balls in the body cavity.

Cercarial embryos develop in the parent redia to a stage at which the mouth and oral sucker become easily recognizable, the oesophagus and the short gut caeca are visible, and the eyes appear as a pair of pigmented spots with two nuclei each. The two lateral ducts of the excretory system are seen to be joined by an arch-shaped anastomosis across the body. At this immature stage, when the length of the body is 0·15–0·19 mm. and of the short tail 0·05–0·08 mm., the young cercariae with their colourless bodies and as yet unrecognizable posterior suckers emerge from the parent rediae (Fig. 14). Cercariae leaving the parent rediae of the first generation were first observed 21 days after exposure of the snail to a miracidium, and snails dissected subsequently were found to be harbouring numerous free-developing cercariae at various stages of development towards maturity.

After leaving the parent redia the young cercaria grows in size, feeding on the snail tissue; when it has almost reached the size of the mature stage the acetabulum becomes well-developed and the tail long, and pigment begins to spread outwards from the eye-spots in irregular dendritic branches over the whole body. At this stage

the excretory bladder, as well as its junction with the tail duct, is clearly visible (Fig. 15). The granules and rods at this stage appear in masses in the cystogenous glands on the surface of the body, and when the cercaria approaches maturity they, in conjunction with the heavy pigmentation of the body, obscure the internal structure.



Paramphistomum phillerouxi n.sp.

Fig. 13.—First-generation redia from a snail dissected 18 days after its exposure to a miracidium. Fig. 14.—Newly born young cercaria. Fig. 15.—Young cercaria developing in snail tissue. Fig. 16 and 17.—Encysted cercaria, dorsal and ventral view of the cyst.

The mature cercaria of *P. phillerouxi* is heavily pigmented, has two dark eye-spots, and is about 0·30 mm. long, the tail being about twice the length of the body.

The first shedding of cercariae by an infected snail was observed 42 days after its exposure to a single miracidium. Despite the comparatively small size of *Bulinus forskalii*, each infected snail shed from 150 to 459 cercariae daily. After swimming for half an hour, the cercariae attached themselves to leaves of plants or to the container wall near the water surface, and encysted.

The cyst of *P. phillerouxi* is round when viewed from above, and dome-shaped in lateral view (Fig. 16 and 17). The slightly flared

base acts as a support and attaches the cyst to the substratum. The diameter of the cyst varies from 0·17 to 0·19 mm. and its base is about 0·20 mm. in diameter. The total height of the cyst is about 0·10 mm.

Summary

(1) *Paramphistomum phillerouxi* sp.nov. from rumina of cattle in Northern Rhodesia is described and its morphology compared with that of *Paramphistomum microbothrium* Fischoeder from Egypt, Kenya, Northern Rhodesia, and South Africa.

(2) *Bulinus forskalii* (Ehrenberg) is a natural intermediate host of *P. phillerouxi* in Northern Rhodesia and Tanganyika. Of other snails, *Bulinus senegalensis* (Müller) from Gambia was successfully infected experimentally, and *Bulinus cernicus* (Morelet) in Mauritius was found naturally infected with *P. phillerouxi*.

(3) The development of *P. phillerouxi* in *Bulinus forskalii* is described. The sporocyst gives birth to 18 to 28 rediae. These first-generation rediae are able to produce daughter rediae as well as cercariae. It seemed that not all of the first-generation rediae started to produce daughter rediae first. Some of the rediae began by producing cercariae and produced a few daughter rediae later alongside numerous cercariae. There is some evidence that the subsequent generations of rediae are also capable of developing daughter rediae and cercariae simultaneously.

(4) The first young cercariae to leave the parent rediae were observed 21 days after exposure of the snails to a single miracidium each, and the first shedding of mature cercariae was noted 42 days after the exposure, the snails being kept at 26°C.

References

Dinnik, J. A. and Dinnik, N. N., 1954.—"The life cycle of *Paramphistomum microbothrium* Fischoeder, 1901 (Trematoda : Paramphistomidae)". *Parasitology*, **44,** 285–99. (W.L. 16035).

Dinnik, J. A., 1954.—"*Paramphistomum sukari* n.sp. from Kenya cattle and its intermediate host". *Ibid.*, **44,** 414–21.

Dinnik, J. A. and Dinnik, N. N., 1957.—"Development of *Paramphistomum sukari* Dinnik, 1954 (Trematoda : Paramphistomidae) in a snail host". *Ibid.*, **47,** 209–16.

Fischoeder, F., 1901.—"Die Paramphistomiden der Saugetiere". *Zool. Anz.*, **24,** 367–75. (W.L. 23833).

————, 1903.—"Die Paramphistomiden der Saugetiere". *Zool. Iber.* (*Syst.*), **17,** 485–660. (W.L. 23835).

Leroux, P. L., 1930.—"A preliminary communication on the life cycle of *Cotylophoron cotylophorum* and its pathogenicity for sheep and cattle". *Rep. vet. Res. S. Afr.*, **16,** 243–53. (W.L. 18640).

————, 1957.—"Report to the Government of the Federation of Rhodesia and Nyasaland on the control of parasitic diseases in livestock". *F.A.O. Report*, **N.696,** 1–47.

Nasmark, K. E., 1937.—"A revision of the trematode family Paramphistomidae". *Zool. Bidr. Uppsala*, **16,** 301–565. (W.L. 23836).

Price, E. W. and McIntosh, A., 1944.—"Paramphistomes of North American domestic ruminants". *J. Parasit.*, **30,** *Suppl.* 9. (W.L. 11428).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 91–100.

Redescription of *Labiduris africana* Gedoelst, 1916 with New Host Records and a Summary of our Present Knowledge of the Genus *Labiduris* Schneider, 1866

By W. M. Fitzsimmons, F.R.C.V.S.
Commonwealth Bureau of Helminthology, St. Albans, Herts.

Labiduris africana Gedoelst, 1916 was collected from two tortoises belonging to the genus *Kinixys*, viz. *K. australis* in Swaziland and *K. belliana* in Nyasaland. It was originally described by Gedoelst from *K. erosa* in the Belgian Congo. A redescription from the new material is followed by a key to the known species of the genus *Labiduris* and a summary and discussion of the somewhat confusing literature on these species.

Labiduridae Thapar, 1925

Labidurinae Yorke and Maplestone, 1926

Labiduris Schneider, 1866

Definition : Labidurinae—mouth with three prominent lips, one dorsal and two subventral ; the subventral are separated from the dorsal by relatively shallow notches, but from one another by a cleft extending to their bases ; the median free edges are curved, overlap each other and are fringed posteriorly ; vestibule short ; oesophagus consists of two parts, the posterior ending in a bulb ; this is followed by a post-oesophageal bulb containing a valvular apparatus. *Male* : two equal spicules, accessory piece and telamon present or absent ; two conical processes on either side of the anus each bearing a papilla at its tip ; an adanal pair of papillae ; three to five pairs of preanal papillae ; four to five pairs of post-anal papillae ; the body ends in a conical pointed tail of varying length in different species. *Female* : tail long and conical ; vulva near anus ; genitalia single throughout ; viviparous.

Type species : *L. gulosa* (Rudolphi, 1819).

Labiduris africana Gedoelst, 1916

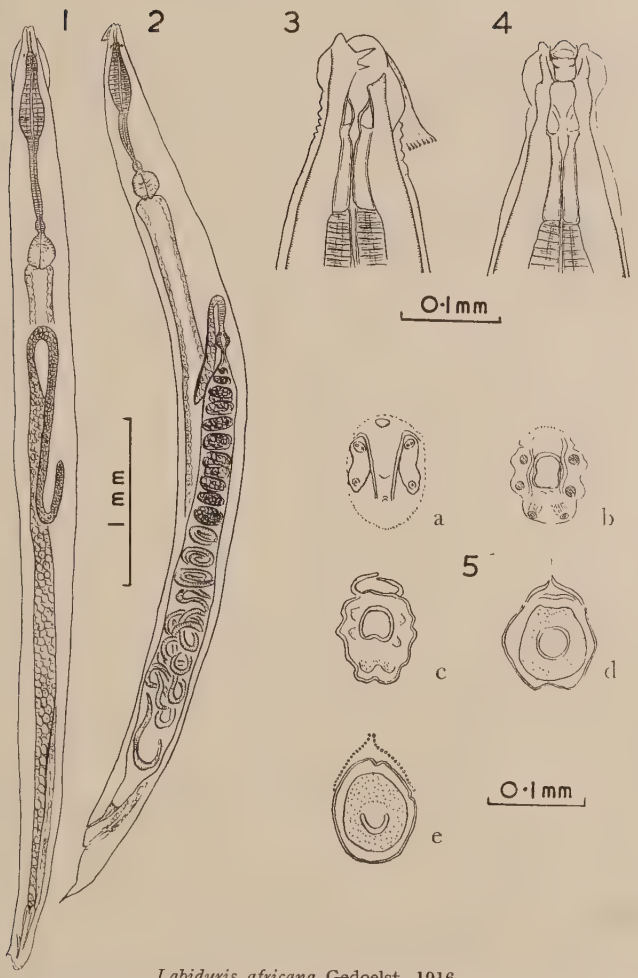
Material containing this species was collected from *Kinixys australis* in Swaziland and from *K. belliana* in Southern Nyasaland and also in Northern Nyasaland near the Tanganyika border.

This material collected from the two species of *Kinixys* from widely separated localities is so similar that there can be no hesitation in declaring it to be identical. A general description is first given which is applicable to both the Swaziland and the Nyasaland material and a separate account of measurements for each is given after this.

Both sexes are represented and very abundant ; adult males and females are present in approximately equal numbers. Only a small proportion of females contain embryos. The males are nearly equal to the females in size. In general appearance this worm is white, elongate and spindle-shaped. The posterior extremity of the male has a truncate appearance. There is a very narrow cuticular crest arising 0·26–0·28 mm. from the anterior extremity and running the length of the body to disappear at the level of the origin of the rectum. This crest is easily seen in the Nyasaland material and is about 10–13μ wide, but it has not been possible to find it in the Swaziland material. Lateral papillae are present on the body wall.

The head is bilaterally compressed and is off-set from the rest of the body by a slight constriction. The mouth is terminal and surrounded by three lips. The dorsal lip has its anterior margin set slightly further back than the margins of the two other lips and it is separated from them only by rather shallow clefts. It is supported by two finger-like structures lying in its depth and bears two simple papillae at the ends of these. It does not have a crenated margin as described by Thapar (1925) for *Labiduris zschokkei*. The two latero-ventral lips are separated from each other ventrally by a wide deep cleft which is closed more posteriorly by a special arrangement consisting of cuticular blades inserted anteriorly on the latero-ventral lips at their median free edges and which can overlap each other and which bear on their posterior margin a fringe of "hairs" tipped with little bead-like knobs. The latero-ventral lips are also provided with two supports each tipped by papillae, the most ventral of the papillae on each lip has a double opening. The dorsal lip bears on its inner surface a flange which projects into the buccal cavity between the latero-ventral lips so that the direction of the mouth opening is diverted somewhat ventrally. Immediately behind this flange there is another small process.

The very thin-walled vestibulum has little crests or lamellae of cuticle projecting into its lumen ; it is followed by a thicker-walled, non-muscular "pharynx" the lumen of which is narrow except at the anterior extremity where it dilates slightly at the junction with the vestibulum. The oesophagus consists of two parts ; an anterior, very muscular part shaped like an elongated pear and a posterior, less muscular part consisting of an isthmus and two bulbs.



Labiduris africana Gedoelst, 1916

Fig. 1.—Mature male. Anterior end twisted to show its ventral surface, posterior end showing lateral surface. Fig. 2.—Mature female, lateral view. Fig. 3.—Head end, lateral view, focussed down through half its depth to show internal structure. Fig. 4.—Head end, ventral view, focussed down through half of its depth to show internal structure.

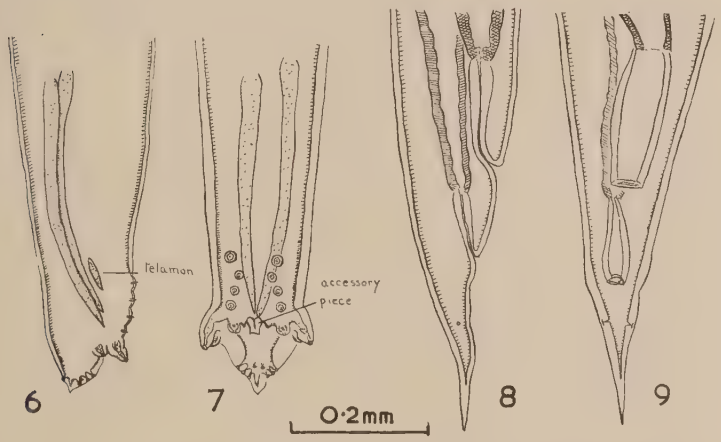
Fig. 5.—Head end, *en face* view at progressive depths of focus starting from most anterior and superficial at (a) through (b), (c) and (d) to most posterior and deep at (e).

The first bulb is smaller than the second bulb and is joined to it by a short neck or constriction. The second bulb contains a valvular and grinding apparatus. The intestine is straight. The nerve ring surrounds the anterior muscular part of the oesophagus in its first quarter. The excretory pore is not easy to discern, but in a few specimens, it is seen behind the level of the oesophageal bulb. It is circular and very small.

The male is comparatively robust, and its posterior extremity has a blunt, rounded appearance when viewed from the dorsal or ventral aspect, and is obliquely truncate when viewed from the side. It terminates in a short mucron which is enveloped in cuticle. Behind the level of the anus the cuticle is inflated ventrally, laterally, and posteriorly, to form caudal alae which are supported by pedunculated papillae. Ventro-laterally on each side of the anus there are two cone-shaped appendices ; a large one somewhat ventral and anterior in position and a smaller one more dorsal and posterior in position, each bearing a papilla on its apex. There are three (occasionally four) sessile preanal papillae arranged symmetrically in two more or less parallel rows on the ventral surface in front of the anus. There is a similar pair of adanal papillae placed ventrally on either side of the anus medial to the appendices. There are five pairs of pedunculated post-anal papillae. One pair is dorsal, three pairs are ventro-lateral and immediately inside these three ventro-laterals there is a minute fifth pair situated ventrally. The anal orifice is a transverse cleft. Its ventral lip is continuous with a transverse fold of cuticle which joins the bases of the adanal papillae and bears a protuberance on either side of the anus internal to these papillae. There is a somewhat chitinised cuticular structure originating from the dorsal wall of the cloaca in midline and protruding beyond the ventral anal lip which possibly functions as an accessory piece. In some individuals a spindle-shaped telamon is visible from the lateral aspect, ventral to the spicules and some distance in front of the anus. There are two cylindrical, rod-shaped, equal spicules which end in a point distally. When viewed from the side they are ventrally curved. They are supported by a hyaline sheath inserted near their tips which may restrict their protrusible length. The male genitalia are single and consist of a coiled testis and a vesicula seminalis.

The female reaches its maximum width at mid-point of the body. The tail is conical, acutely pointed, and laterally, behind the anus, the cuticle is dilated and supported by a single small papilla on either side. The vulva is situated only a short distance in front of the anus and its lips protrude very slightly. A short, thick-walled vagina leads directly into a capacious thin-walled uterus which in reproducing

individuals is filled with embryos posteriorly, and eggs in progressive stages of development anteriorly. Anteriorly the single uterus narrows suddenly and connects with a spherical ovejector which is, in turn, connected to the ovary by a narrow oviduct which at first runs anteriorly and is then reflected posteriorly to join the narrow ovary.



Labiduris africana Gedoelst, 1916

Fig. 6.—Posterior end, male, lateral view. Fig. 7.—Posterior end, male, ventral view. Fig. 8.—Posterior end, female, lateral view. Fig. 9.—Posterior end, female, ventral view.

Specific diagnosis :

(a) Swaziland material.

Male : Length, 3·57–6·5 mm.; average 4·92 mm.; greatest width, 0·28–0·42 mm.; average 0·35 mm.; length pharynx, 0·07–0·08 mm.; average 0·074 mm.; length anterior part of the oesophagus, 0·31–0·32 mm.; length posterior part of the oesophagus, plus two bulbs, 0·56–0·58 mm.; maximum width of anterior part of oesophagus 0·10–0·12 mm.; average width narrow posterior part of oesophagus, 0·041 mm.; width first bulb, 0.06–0·07 mm.; width second or true oesophageal bulb, 0·14–0·16 mm.; head-nerve ring distance, 0·20 mm.; anus-tail distance, 0·083–0·084 mm.; length spicules, 0·26–0·30 mm., average 0·28 mm.

Female : Length, 4·0–7·1 mm.; average 5·6 mm.; greatest width 0·26–0·50 mm.; average 0·32 mm.; pharynx length, 0·076–0·086 mm.; average 0·081 mm.; length anterior part of oesophagus, 0·32–0·33 mm.; length posterior part of oesophagus plus two bulbs, 0·58–0·60 mm.; greatest width anterior part of oesophagus, 0·10–0·12 mm.; average width narrow posterior part of oesophagus, 0·053 mm.; width first bulb, 0·07 mm.; width second or true bulb, 0·15–0·16 mm.; head-nerve ring distance, 0·25 mm.; anus-tail tip distance, 0·23–0·37 mm., average 0·31 mm.; vulva anus-distance, 0·17–0·20 mm., average 0·19 mm.

(b) Nyasaland material.

Male : Length, 4·45–7·0 mm.; average, 6·0 mm.; greatest width, 0·38–0·50 mm.; average, 0·44 mm.; length pharynx, 0·070–0·080 mm.; average, 0·076 mm.; length anterior part of oesophagus, 0·29–0·32 mm.; average, 0·30 mm.; length posterior part of oesophagus plus two bulbs, 0·54–0·65 mm.; average, 0·60 mm.; maximum width anterior part of oesophagus, 0·12–0·14 mm.; average width narrow posterior part of oesophagus, 0·05 mm.; width first bulb, 0·07 mm.; width second or true oesophageal bulb, 0·15–0·16 mm.; head-nerve ring distance, 0·21 mm.; anus-tail tip distance, 0·07–0·08 mm.; average 0·076 mm.; length spicules, 0·27–0·30 mm.; average, 0·29 mm.

Female : Length, 5·6–7·0 mm.; average 6·6 mm.; greatest width 0·48–0·52 mm.; average, 0·51 mm.; length pharynx, 0·078–0·084 mm.; average, 0·080 mm.; length anterior part of oesophagus, 0·30–0·34 mm.; average 0·32 mm.; length posterior part of oesophagus plus two bulbs, 0·59–0·60 mm.; greatest width anterior part of oesophagus, 0·12–0·13 mm.; average width narrow posterior part of oesophagus, 0·054 mm.; width first bulb, 0·07 mm.; width second or true bulb, 0·16 mm.; head-nerve ring distance, 0·26 mm.; anus-tail tip distance, 0·30–0·38 mm.; average, 0.33 mm.; vulva-anus distance, 0·23–0·24 mm.

Hosts and Localities : *Kinixys australis* (Hewitt),
Bremersdorp, Swaziland.

Kinixys belliana (Gray),
Fort Hill, Northern Nyasaland and Blantyre, Southern Nyasaland.

Habitat : Large intestine and caecum.

Key to the Species of the Genus Labiduris

1. Lateral alae present, excretory pore post-bulbar..... .....*L. africana* Gedoelst, 1916

— Lateral alae absent, excretory pore pre-bulbar.....2

2. Caudal process of male long and subulate ; pharynx comparatively short ; excretory pore at level of junction of small and large oesophageal bulbs ; cuticular barbs on ventral cloacal lip present.....*L. gulosa* (Rud., 1819) Schneider, 1866

— Caudal process of male short and spike-like ; pharynx comparatively long ; excretory pore half-way along isthmus between first part of oesophagus and posterior bulbs ; no barbs on ventral cloacal lip.....*L. zschokkei* Linstow, 1899

Affinities, Notes and Discussion on the genus *Labiduris* Schneider, 1866

The generic diagnosis by Yorke and Maplestone (1926) has been altered slightly to include the occasional presence of an accessory piece (Thapar, 1925) and the telamon and the extra pair of postanal papillae recorded in this paper.

The species recorded above from new hosts in Swaziland and Nyasaland resembles very closely both *Labiduris zschokkei* von Linstow, 1899 and *Labiduris africana* Gedoelst, 1916.

The literature on the genus *Labiduris*, although not plentiful, is somewhat controversial and it is thought appropriate to give a brief outline of it here.

Rudolphi (1819) described a new species *Ascaris gulosa*, from the Brazilian land tortoise, *Testudo tabulata*. Diesing (1851) recorded the same species from several other hosts, *Chelonoides tabulata*, *Rhinemys nasuta* and *Testudo graeca* from which it has never been recorded since and, it is felt, that these records, in particular the one from *T. graeca* must be regarded with doubt, more especially since the parasites of the latter tortoise have since been so frequently and thoroughly investigated. Schneider (1866) erected the genus *Labiduris* for the reception of Rudolphi's species.

Von Linstow (1899) gave a generic diagnosis and described a new species, *L. zschokkei*, from *T. tabulata*. He showed that the main points of difference between *L. gulosa* and *L. zschokkei* are the following:

(a) tail long in *L. gulosa*, short in *L. zschokkei* ; (b) one papilla outside either submedian preanal row in the former, two in the latter. He also mentioned, for *L. zschokkei*, the presence of somatic papillae over the whole body, a total of 24 genital papillae on the posterior extremity of the male, i.e. five preanal pairs, one adanal pair, one papilla on each of the four lateral processes, and four pairs of postanal papillae. Of the preanals three are in each submedian row and two papillae are outside each row. He reported the spicules to be equal and 0·48 mm. long. The excretory pore is said to be a distance of 3/11 the oesophagus length behind the oesophageal bulb, i.e. post-oesophageal position.

Chapin (1924) examined new material from *T. tabulata* and reported a great range of variation in the length of the spicules, length of tail and position and number of the preanal papillae. He found that the spicule length varied from 0·48 mm. to 0·60 mm. and that the preanal papillae may be as many as five in each of the submedian rows with either none, one, or two on either side of the rows. He did not consider that the points of difference between *L. gulosa* (Rudolphi) as described by Schneider and *L. zschokkei* Linstow, as brought out by von Linstow's original description, held in a large series and he placed von Linstow's species in synonymy with *L. gulosa* (Rudolphi).

Thapar (1925) after a study of material from *T. tabulata* came to the conculsion that the range in variation in number of precloacal papillae does exist as stated by Chapin (1924) but that the differences are well marked in the length of the tail in the two forms. He redescribed both species and showed that they could be distinguished from each other by tail length, relative length of pharynx, position of excretory pore, relative distance between anus and vulva and the presence (in *L. gulosa*) and the absence (in *L. zschokkei*) of cuticular barbs on the ventral cloacal lip. He stated that the excretory pore of *L. zschokkei* is prebulbar in position. He confirmed the presence of somatic papillae in *L. zschokkei* and showed a pair of lateral caudal papillae in the female. He did not mention the presence of bead-like knobs at the ends of the fringes on the latero-ventral lips recorded by von Linstow. He does not give a detailed account of the genital papillae of the male of *L. zschokkei*, merely stating that the "genital papillae are of the common type and have the usual disposition of parts". However, in his figure 131, posterior end of male, ventral view, he shows three ventro-lateral pedunculated postanal papilla on either side and internal to them a small pedunculated ventral papilla. If it can be assumed that the dorsal pedunculated postanal papilla is out of sight in this figure, then his specimens of *L. zschokkei*

must have had a total of five pairs of pedunculated postanal papillae. He placed *L. africana* Gedoelst, 1916 in synonymy with *L. zschokkei* Linstow, 1899, but considered that the latter and *L. gulosa* (Rudolphi, 1819) were distinct species.

Gedoelst (1916) reported the finding for the first time of a member of the genus from an African tortoise, *Kinixys erosa*, taken in Leopoldville, Belgian Congo, and named it *Labiduris africana*. He mentioned, amongst other characters, the presence of lateral flanges (lateral alae) and the postbulbar position of the excretory pore and the presence of a pair of single papillae on each of the lips surrounding the mouth. He stated that there were four pairs of sessile preanal papillae in the male disposed in two longitudinal rows and four pairs of postanal papillae. The length of the equal spicules is given as 400 to 415μ. He did not report the presence of somatic papillae or of a pair of caudal papillae in the female.

From the new records of African *Labiduris* species from the *Kinixys* tortoises it will be seen that there are more similarities between them and the Brazilian *L. zschokkei* than have heretofore been recorded. Thus the following additional points in common have been noted : the presence of somatic papillae, five pairs of pedunculated postanal papillae in the male. In agreement with von Linstow and in disagreement with Thapar—the post-oesophageal position of the excretory pore and the presence of bead-like knobs tipping the fringe of the latero-ventral lips. In agreement with Thapar, the presence in the female of a pair of lateral caudal papillae half-way between the anus and the tip of the tail. Points of difference are that there has never been a record of extra pre-anal papillae outside the submedian rows in African material and the presence in Gedoelst's material and in the material from Nyasaland, of distinct lateral alae.

Although Brazilian material has been well examined by a number of distinguished investigators there has never been a record of the presence of lateral cuticular alae in *L. zschokkei*, the species which appears to have the closest affinity with Gedoelst's *L. africana*. Also, if Thapar (1925) was correct, the position of the excretory pore is different in the two species. Consequently, although it is apparent that the two species are very closely related, it is felt that until further study of the Brazilian material can be undertaken and because of the wide geographical separation, Gedoelst's *L. africana* should be upheld as valid.

Geographical distribution : Of the three species of the genus *Labiduris* two (*L. gulosa* and *L. zschokkei*) are recorded only from *Testudo tabulata* (*Testudo denticulata*) which inhabits the equatorial

jungle areas of South America. The only definite record of the place of origin of a specimen from which worms were collected is given by Chapin (1924) who obtained his material from tortoises collected at Para, Brazil. Gedoelst (1926) recorded *L. africana* from the hinged land tortoise, *Kinixys erosa*, collected at Leopoldville on the Congo river. In the present paper the same species is recorded from the Northern and Southern limits of Nyasaland and from Swaziland, again from hinged tortoises of the genus *Kinixys*. It is noteworthy that no *Labiduris* has been found in tortoises of other genera found further south than Swaziland and it would seem that its distribution coincides with that of the genus *Kinixys* in Africa (in South Africa the distribution of *Kinixys* is limited to Bechuanaland, Transvaal, and Zululand according to Rose). No *Labiduris* were ever found in a number of *Testudo pardalis* collected from the same locality as *Kinixys australis* in Swaziland.

Acknowledgment

The author wishes to express his gratitude to the Director of the Commonwealth Bureau of Helminthology for permission to publish this paper.

References

Chapin, E. A., 1924.—"Nematode Parasites of the Brazilian Land Tortoise, *Testudo denticulata*." *Proc. U.S. nat. Mus.*, **65,** 1–6. (W.L. 16944).

Diesing, K. M., 1851.—Systema Helminthum. Berlin, **2,** 588 pp.

Gedoelst, L., 1916.—"Notes sur la faune parasitaire du Congo Belge." *Rev. zool. afr.*, **5,** 27–33. (W.L. 19323).

Linstow von, O., 1899.—"Nematoden aus der Berliner zoologischen Sammlung." *Mitt. zool. Mus. Berlin*, **1,** 1–28. (W.L. 14243).

Rose, W., 1950.—The Reptiles and Amphibians of Southern Africa. Maskew Miller Ltd., Cape Town. p. 346.

Rudolphi, C. A., 1819.—Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. 811 pp.

Schneider, A., 1866.—Monographie der Nematoden. Berlin, 357 pp.

Thapar, G. S., 1925.—"Studies on the Oxyurid Parasites of Reptiles." *J. Helminth.*, **3,** 83–150. (W.L. 11224c).

Yorke, W. and Maplestone, P. A., 1926.—The Nematode Parasites of Vertebrates. J. & A. Churchill, London.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 101–108.

On the Migration Route of the Infective Larvae of *Nippostrongylus brasiliensis**

By H. M. Gharib, M.R.C.V.S., Ph.D.

*From the Department of Parasitology, London School of Hygiene and Tropical Medicine***

In 1922 Yokogawa described in great detail the life cycle, the free-living larval stages, methods of culturing the larvae, infection and migration in the host as well as the morphology of the adult parasite *Nippostrongylus brasiliensis* (Syn. *Heligmosomum muris*). He stated that the larvae of this parasite penetrate the skin into the tissues and travel to the lungs, ". . . by means of blood stream." Taliaferro and Sarles (1939) studying the skin of rats during the initial infection with these larvae stated that "The third stage of inflammation was initiated at sixteen hours by the movement of the larvae toward and through the larger subcutaneous veins during which small haemorrhages occurred . . .". Barakat (1948) stated that, "The larvae after penetrating the skin are carried by the lymph or blood circulation to the lungs". The writer (1955) in trying to ascertain the sites likely to be affected by bacteria introduced by the infective larvae of this parasite, while investigating the role of tissue invading helminths in bacterial infections, found that it was necessary to confirm the observations of the above mentioned investigators and with this end in view the following experiments were carried out.

Material and Methods

Albino rats and mice of ages varying from three weeks to two months, which has been proved by previous repeated examinations of their faeces to be free from *N. brasiliensis* infection, were used in the experiments. The animals (mouse or rat) were infected by placing them in a glass jar of suitable size, containing infective larvae in a few c.c. of filtered water. By this method of exposure the larvae penetrate mainly through the skin of the hind limbs, especially between the claws, and as the body gets wet the animal usually licks its skin and infection also takes place through the mouth. Infective larvae were obtained by Barakat's technique (1951).

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

**Present address: Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Cairo.

Experiments

Experiment No. 1

Fourteen rats were exposed to infection in a glass jar for one hour, after which time the rats were removed to clean cages. It was arranged that one animal was anaesthetized and secured in position, abdomen up, at hourly intervals from one to fourteen hours. The abdominal skin of each rat was dissected and the blood vessels of the axilla were severed. The blood which oozed into the axillary space was sucked by a glass pipette into a test tube containing 5 c.c. of normal saline solution and then maintained at 28 C. for a quarter of an hour and the supernatant fluid was examined by a hand lens (X 12) and afterwards under a binocular microscope in a Petri dish. The blood precipitate was then examined under a binocular microscope between two slides. The abdominal and thoracic cavities were opened separately and washed with normal saline which was transferred to a Petri dish and examined for the presence of larvae. The liver of each animal was cut into pieces and each piece flattened between two slides and examined under the dissecting microscope for the presence of larvae. This experiment was repeated on three occasions using fourteen albino mice.

Result : No larvae were recovered from the blood, abdominal and thoracic cavities nor from the liver of the exposed animals.

Experiment No. 2

Fourteen mice were exposed per rectum to infective larvae and very large numbers of larvae were added to a beaker containing 10 c.c. of filtered water. Each mouse was lightly anaesthetized and held up by its tail. The anal region was rubbed with glycerine and by means of an eye-dropper, 0·5 c.c. of the larval suspension from the beaker was introduced into the rectum of each mouse. The mouse was kept held from the tail for about five minutes after which time the anal region was wiped and the animal was kept in a glass jar. One animal was killed at hourly intervals from one to fourteen hours after exposure. The liver of each animal was cut into pieces and each piece flattened between two slides and examined under the low power of the microscope.

Result : No larvae were found in the liver of any of the mice.

Experiment No. 3

Two rats were inoculated intravenously, via the coccygeal vein with about 200 infective larvae in filtered water. Another rat was

infected by skin to act as a control. The three rats were killed 24 hours after exposure to infection and the lungs were examined for the presence of larvae.

Result: No larvae were recovered from the two rats infected intravenously while larvae were recovered from the control, infected via the skin.

On failing to recover the parasite from the liver, blood, or body cavities of infected animals, the lymphatic glands were next examined for the presence of larvae.

Table I

Hours after Exposure	Lymph Glands					
	Axillary	Precrural	Popliteal	Submaxillary	Parotid	Prepectoral
1	—	—	—	—	—	—
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—
7	—	—	—	—	—	—
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	—	—	—	—	—	—
11	—	—	—	—	—	—
12	+	—	—	—	—	—
13	+	—	—	—	—	—
14	+	—	—	—	—	—

Experiment No. 4

Fourteen young mice, three weeks old, were exposed to infective larvae by the application of the parasite to the intact abdominal skin. One animal was killed at one hour, two hours and so on until the fourteenth hour after exposure. To facilitate examination, these animals were not all exposed at the same time. The subcutaneous lymph glands, the axillary, precrural, popliteal, submaxillary, parotid and prepectoral lymph glands were examined between 2 slides under the low power of the microscope.

Result: Twelve hours after exposure, larvae were recovered from the axillary lymph glands (see Table I). No larvae were recovered before this period had elapsed.

Experiment No. 5

Further experiments were conducted to prove or disprove the above findings using 21 white mice which were exposed to infection as in Experiment 4. Three mice were killed after twelve hours, three more an hour later and the rest in groups of three at the 14th, 15th 16th, 17th, and 18th, hours after exposure. The axillary lymph glands and the lungs of all these animals were examined for the presence of larvae.

Result : Larvae were present in the axillary lymph glands in animals killed 12, 13, 14 ,15, 16, and 17 hours after exposure but none were present in the lungs. In the group killed 18 hours after exposure, larvae were recovered from the lungs of all animals but not from the axillary lymph glands (see Table II).

Experiment No. 6

Eight albino mice about four months old were secured abdomen upwards. The skin of the thigh of the right limb was damped with water. One hundred infective larvae on a piece of filter paper were applied to a selected site of exposure in each animal. The time for exposure was one hour after which the animals were released. Twelve, sixteen, eighteen and twenty hours after exposure two animals were killed successively and the precrural lymph glands, popliteal lymph glands of the limb exposed to infection, as well as the lungs of each animal, were examined under the microscope for the presence of larvae.

Result : Larvae were recovered from the precrural and popliteal lymph glands in the animals killed after 12 and 16 hours but none were recovered from the lungs. In the remaining animals no larvae were recovered from the precrural or popliteal lymph glands but larvae were present in the lungs (see Table III).

Experiment No. 7

Seven mice of ages varying between two and three months were used. All animals were lightly anaesthetized and a piece of filter paper bearing about 200 larvae was introduced into the buccal cavity of each mouse. Eight hours after exposure one animal was killed and two hours later another one was killed and so on till the twentieth hour after exposure. The submaxillary and parotid lymph glands and the lungs of each animal were pressed between slides and examined under the low power of the microscope for the presence of larvae.

Result : The parasite was present in the lymphatic glands 12, 14,

and 16 hours after exposure. In the lungs the parasite was present from the 18th hour onwards, as illustrated in Table IV.

Table II

Hours after exposure	Number of animals used	Result of examining the	
		Axillary lymph gland	Lung
12	3	+	—
13	3	+	—
14	3	+	—
15	3	+	—
16	3	+	—
17	3	+	—
18	3	—	+

Table III

Hours after exposure of the hind limb	Number of animals used	Result of examining the		
		Lymph gland		Lung
		Precrural	Popliteal	
12	2	+	+	—
16	2	+	+	—
18	2	—	—	+
20	2	—	—	+

Table IV

Time of examination after exposure	Lymph Glands		Lung
	Submaxillary	Parotid	
8 hours	—	—	—
10 ,,	—	—	—
12 ,,	+	+	—
14 ,,	+	+	—
16 ,,	+	+	—
18 ,,	—	—	+
20 ,,	—	—	+

Experiment No. 8

Thirty-two mice about four weeks old were divided into four equal groups. All animals proved by previous repeated examinations to be free of the parasite *N. brasiliensis*. Each group was exposed to

infection in the following manner : Group 1 per rectum, Group 2 subcutaneously, Group 3 intraperitoneally, and Group 4 intrathoracically. Each group was exposed to a large number of infective larvae. Ten hours after exposure one animal from each group was killed and subsequently an animal from each group was killed at two-hourly intervals, i.e., at 10, 12, 14, 16, 18, 20, 22, and 24 hours after exposure. The lymph glands draining the site of exposure and the lungs of all the animals were pressed between slides and examined under the microscope for larvae to note the time of arrival in these organs.

Result : The result of examinations as summarized in Table V were as follows :

In Group 1 larvae were present in the mesenteric lymph glands 12, 14, and 16 hours after exposure. Larvae first arrived in the lungs 18 hours after exposure.

In Group 2 larvae first arrived in the axillary lymph glands 12 hours after injection and were present in the glands from the 12th to the 16th hour after exposure. Larvae first arrived at the lungs 18 hours after exposure and were present from 18 to 24 hours.

In Group 3 larvae first arrived in the mesenteric lymph glands in 18 to 20 hours. In the lungs larvae first arrived 20 hours after exposure and were present 20, 22, and 24 hours after injection.

In Group No. 4 larvae were first recovered in the bronchial lymph glands fourteen hours after injection and were present from 14 to 18 hours after injection. They first arrived in the lungs 24 hours after injection.

Discussion and Conclusion

Some experiments were conducted on the migration route of the parasite *N. brasiliensis*. The blood and liver of the infected animals were examined and no larvae were found there, but larvae were present in the lymph glands. The reason that the parasites prefer to travel via the lymphatic system may possibly be that the current in the blood stream is too fast and blood contains strong defensive mechanisms which would harm the larvae.

In experiment No. 3 the larvae were injected intravenously, and no trace was found of them in the lungs, on examination, 24 hours after injection. When the infective larvae were injected intrathoracically they did not proceed to the lungs but were recovered

from the bronchial lymph glands first. It may also be reasonable to say that the parasite after reaching the body needs to have a rest during which it feeds, grows and gets acclimatized to the new surroundings before it proceeds to the lungs. This might explain why the parasite is recovered from the lungs about 18 hours from the time of exposure to infection. If it is true that the parasite carries on its surface and inside its body some micro-organisms, during its migration via the blood, it would be very dangerous to the host as the bacteria will multiply very quickly in the blood and cause death. Its migration via the lymph will combat many of these organisms. The fact that sometimes more than thirty larvae were seen in one lymph gland suggest that the lymph is the route of migration. It is of interest to

TABLE V

No. of Group	Method of exposure	Time needed by the larvae for: Arrival in lymph gland	Recovery in lymph gland	Arrival in the lung
1	Per rectum	12 hours	12–16 hours	18 hours
2	Subcutaneously	12 ,,	12–16 ,,	18 ,,
3	Intraperitoneally	18 ,,	18–20 ,,	20 ,,
4	Intrathoracically	14 ,,	14–18 ,,	24 ,,

note here that Standen (1952) demonstrated that the cercariae of *Schistosoma mansoni* penetrate the lymphatic system of the mouse within twenty minutes. It was noticed that all larvae did not travel to the lymph glands or the lungs at the same time. It was observed that when the host was infected through the intact skin or injected sub-cutaneously the larvae reached the nearest lymphatic glands in 12 hours and the lungs in 18 hours, but when they were injected intraperitoneally or intrathoracically they took a longer time to reach the lymph glands draining those areas. The arrival in the lungs was also prolonged compared with the time needed to reach them from the skin. This suggests that the infective larvae might need a longer time to penetrate through the serous tissues than that needed to penetrate the skin. When sections of infected lymph glands of rats, mice and guineapigs were studied, the larvae were noticed to be always present at the periphery of the glands.

From the results of all the above mentioned experiments it seems reasonable to conclude that the infective larvae of *N. brasiliensis* migrate to the lungs of the host via the lymphatic system and not by the blood stream as stated by Yokogawa (1922), Taliaferro and Sarles (1939), and Barakat (1948).

Summary

Some experiments were carried out on the migration route of the parasite *N. brasiliensis*. The infective larvae migrate to the lungs via the lymphatic system and not by the blood stream. The larvae reached the lymphatic gland draining the site of infection from 12 to 17 hours after normal exposure to infection and the lungs from 18 hours onwards.

Acknowledgment

The writer records his thanks to Professor J. J. C. Buckley and to Dr. P. L. LeRoux for their valuable aid and criticism and suggestions during the preparation of this work.

References

Barakat, M. R., 1948.—"Relationship between nutrition and parasitism in the experimental animal." *Ph.D. Thesis of the University of London.*

———1951.—"A new procedure for the cultivation of the nematode parasites." *J. Egypt. Med. Ass.*, **34**, 323-326. (W.L.11172 a).

Gharib, H. M., 1955.—"Some observations on the transmission of bacteria by infective larvae of *Nippostrongylus brasiliensis*." *J. Helminth.*, **29**, 27-32 (W.L. 11224 c).

Standen, O. D., 1952.—"The penetration of cercariae of *Schistosoma mansoni* into the skin and lymphatics of the mouse." (Demonstration) *Trans. Roy. Soc. trop. Med. Hyg.*, **46**, 384. (W.L. 21671).

Taliaferro, W. H. and Sarles, M. P., 1939.—"The cellular reaction in the skin, lung and intestine of normal and immune rats after infection with *N. muris*." *J. inf. Dis.*, **64**, 157-192. (W.L. 11250).

Yokogawa, S., 1922.—"The development of *Heligmosomum muris* Yokogawa, a nematode from the intestine of the wild rat." *Parasitology*, **14**, 127–166. (W.L. 16035).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 109–114.

Some Observations on the Behaviour of *Nippostrongylus brasiliensis* in different kinds of Laboratory Animals*

By H. M. Gharib, M.R.C.V.S., Ph.D.**

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

Experiments

Longevity of N. brasiliensis *in different hosts* :

For this experiment the following animals were used in groups of three : albino rats, white mice, voles, guineapigs and chickens of ages varying from three to four weeks. Faecal examinations of these animals for *N. brasiliensis* infection had proved them to be worm free. To infect the animals they were secured in position abdomen upwards and each animal was exposed to about 200 infective larvae, six days old, for a period of one hour after which time they were released and each group kept in a separate cage. From three days after exposure the faeces of each group were examined daily for the presence of eggs.

Results

The albino rats began to pass eggs five days after exposure and ceased passing eggs one hundred days after exposure. The mice began to pass eggs six days after exposure and seven days later ceased to pass eggs in the faeces. The hamsters were found to pass eggs six days after exposure and four weeks after exposure eggs ceased to be passed. In the cotton rats very few eggs were passed seven days after exposure and ceased to occur from four days later. The chickens, voles, and guineapigs did not pass any eggs at all. (See Table I).

Route of migration of infective larvae in different hosts :

For this experiment the following animals were used in groups of fourteen : albino rats, albino mice, cotton rats, hamsters, voles and guineapigs of ages varying from three to six weeks. Animals were exposed to a large number of infective larvae in a glass jar of

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

** *Present Address* :—Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Cairo.

suitable size. To facilitate exposure and examination the animals were not all exposed to infection at the same time. Infective stage larvae six days old were used for the infection of these experimental animals.

TABLE I

Animal	Date after exposure when eggs were passed	Date after exposure when eggs ceased to be passed
Albino rats	5 days	100 days
Albino mice	6 days	13 days
Hamsters	6 days	28 days
Cotton rats	7 days	11 days
Voles	Nil	Nil
Guineapigs	Nil	Nil
Chicken	Nil	Nil

TABLE II

Animal	Degree of pigmentation of the cells of the intestinal walls of larvae 18 hours after exposure: Lymph Glands	Lungs
Rat	Faintly pigmented	Pigmented
Mouse	Faintly pigmented	Pigmented
Guineapig	Highly pigmented	Highly pigmented
Vole	Highly pigmented	Highly pigmented
Cotton rat	Pigmented	Highly pigmented
Hamster	Pigmented	Highly pigmented

TABLE III

Animal	Presence of larvae 24 hours after exposure in: Lymph Glands	Lungs	Small Intestine
Rat	Nil	Positive	Nil
Mouse	Nil	Positive	Nil
Cotton rat	Positive	Positive	Nil
Hamster	Positive	Positive	Nil
Vole	Positive	Positive	Nil
Guineapig	Positive	Positive	Nil

Two animals were killed from each group 12, 18, 24 and 70 hours, and one and two weeks after exposure. The lymphatic glands, lungs and small intestines were examined. Sections were prepared from the precrural lymphatic glands of rats 12 hours after exposure and from the same glands of the exposed guineapigs 48 hours after exposure.

Results

(1) Twelve hours after exposure larvae were present in most of the lymphatic glands of the experimental animals but not in the lungs or intestines. In the sections, it was noticed that the larvae were to be found mostly in the cortex of the lymph glands of rats without or with very slight inflammations around them (Fig. I).

(2) Eighteen hours after exposure larvae were present in most of the lymphatic glands of all the experimental animals as well as in the lungs, but not in the alimentary canal. The number of larvae in the lymphatic glands of the rat or the mouse 18 hours after exposure to infection was very small or negligible compared with the large numbers in other experimental animals. The cells of the intestinal walls of the parasites in the lymph glands were observed to be pigmented differently in different host groups as follows :—

(a) Faintly pigmented in rats and mice, if present.
(b) Well pigmented in cotton rats and hamsters.
(c) Highly pigmented in guineapigs and voles.

In the lungs the cells of the intestinal walls of the larvae were observed to be highly pigmented in all the experimental animals exposed to infection except the rats and mice in which they were not so markedly pigmented (Table II).

(3) Twenty-four hours after exposure larvae were present in the lymph glands of cotton rats, hamsters, voles and guineapigs but not in those from the rats or the mice. Larvae were present in the lungs of all the animals (Table III). No larvae were recovered from the small intestine of any of the animals.

(4) The result of examination of the lymph glands, lungs and small intestines 48 hours after exposure was the same as for 24 hours. In the sections made from the lymph glands of the guineapigs the parasite was seen to be surrounded by a zone of inflammation 48 hours after exposure (Fig. 2).

(5) Seventy hours after exposure larvae were present in the lymph glands of cotton rats, hamsters, voles, and guineapigs but not in the lymph glands of rats or mice. Larvae were present in the lungs of all the animals seventy hours after infection (Table IV). Larvae were present in the small intestine of the rats, the mice, the cotton rats and the hamsters, but not in the voles or the guineapigs.

(6) One week after exposure dead larvae were present in the lymph glands of the guineapigs and the vole, but not in the lymph glands of the other animals (Table V). The larvae were present in the lungs of the cotton rats, hamsters, voles and guineapigs, but not in the lungs of rats or mice. The parasite was recovered one

week after exposure from the small intestine of the rats and mice and recovered stunted in the hamsters and cotton rats and was not recovered from the voles or the guineapigs.

TABLE IV

Animal	Presence of larvae 70 hours after exposure in Lymph Glands	Lungs	Small Intestine
Rat	Nil	Positive	Positive
Mouse	Nil	Positive	Positive
Cotton rat	Positive	Positive	Positive
Hamster	Positive	Positive	Positive
Vole	Positive	Positive	Nil
Guineapig	Positive	Positive	Nil

TABLE V

Animal	Presence of larvae one week after exposure in Lymph Glands	Lungs	Small Intestine
Rat	Nil	Nil	Positive
Mouse	Nil	Nil	Positive
Cotton rat	Nil	Positive	Positive
Hamster	Nil	Positive	Positive
Vole	Positive	Positive	Nil
Guineapig	Positive	Positive	Nil

TABLE VI

Animal	Presence of larvae two weeks after exposure in Lymph Glands	Lungs	Intestinal Tract
Rat	Nil	Nil	Positive
Mouse	Nil	Nil	Nil
Cotton rat	Nil	Positive	Nil
Hamster	Nil	Positive	Positive
Vole	Nil	Positive	Nil
Guineapig	Positive	Positive	Nil

(7) Two weeks after exposure few dead larvae were present in the lymph glands of the guineapigs but none were present in the lymph glands of the other animals (Table VI). The examination of the lungs of the animals revealed that no larvae were present in the rats or the mice while in the lungs of the voles, hamsters, cotton rats and guineapigs dead larvae were present. No parasites were recovered from the intestinal canal of the mice, cotton rats, voles or guineapigs, but they were present in this organ in rats and hamsters.

(8) During the macroscopic examination of the infected lymphatic glands of the different experimental animals it was noticed that these glands were congested in the guineapigs, voles, cotton rats and hamsters while those of the rats and mice appeared to be normal in colour.

(9) Raised haemorrhagic spots were noticed on the undersurface of the skin in the animals killed after the 18th hour of exposure. A marked congestion of the tissues around the superficial lymphatic glands of the body was also observed (Fig. 3). Twenty-four hours after exposure the spots on the undersurface of the skin and the congestion around the lymph glands were noticed to subside and at 48 hours the congestion had disappeared.

Discussion and Conclusion

From the results of these studies it seems that the rat is the most specific host for the parasite *N. brasiliensis*. The parasite reaches maturity and passes eggs five days after exposure to infective larvae. Infection may carry on for a hundred days or more. The mouse and hamster come next to the rat with regard to infectivity. The life span of the parasite in these two animals is much shorter than in the rat. The cotton rat is readily infected but the number of eggs passed is very small and it is doubtful if these eggs will ever hatch to the infective stage. The voles and guineapigs seem to contract infection but they are not specific hosts. Chickens are not at all liable to infection to this parasite. This might be due to the high temperature of birds which might lead to death of larvae. The cells of the intestinal wall of the larvae in the lymph glands are more highly pigmented in unspecific hosts than in the rat and mouse. This high pigmentation might be due to the great reaction of the body tissues to the parasites. Gharib (1961) conducted some experiments on the migration route of the infective larvae of *N. brasiliensis* in rats and mice and it was proved that this parasite migrated inside the body to the lungs via the lymphatic system. The route of migration appears to be the same in other laboratory animals, a fact which strengthens the idea of migration via the lymphatic system and not via the blood system. Twelve hours after exposure to infection, larvae were recovered from the lymph glands and 24 hours after exposure, larvae were recovered from the lymph glands of the cotton rat, hamster, vole and guineapig but not from the rat nor from the mouse. At 70 hours after infection the larvae were still present in the lymphatic glands of the cotton rat, hamster, vole and guineapig, but after one week they were only seen, dead, in lymph glands of

the vole and guineapig. After two weeks larvae were still found dead in the lymph glands of guineapigs only. The parasite reaches the lungs of all experimental animals 18 hours after infection, but it does not proceed further than the lung in the vole and guineapig. Thus, it seems that these animals, vole and guineapigs, are not specific hosts. It is an interesting observation that animals other than specific hosts, may be attacked by these parasites through the skin. But, as the parasites do not mature and penetrate only as far as the lymph glands and the lungs, they cause no harm to the animal. In such a case, the presence of the parasite and its associated bacteria may either give specific immunity or may set up an infection, which could possibly lead to disease or death of the host, the point being that it is difficult to detect the presence of the parasite in the lymph glands or lungs.

Summary

(1) Rats and mice are specific hosts to *N. brasiliensis*.

(2) The life span of the parasite may reach up to 100 days in the rat but is much shorter in other experimental animals.

(3) Chickens do not become infected with this parasite.

(4) It was found that the route of migration of this parasite inside the body to the lungs was via the lymph system and not via the blood in all experimental animals.

(5) The larvae, after application to the skin, reach the lymph glands draining the site of infection, not before 12 hours ; and they reach the lungs at 18 hours after exposure to infection.

(6) The larvae did not progress further than the lungs in abnormal hosts such as the guineapig and the vole.

(7) The cells of the intestinal canal of the larvae, either in lymph glands or lungs, are highly pigmented in unspecific hosts.

(8) The larvae during their presence in the lymph glands prefer to stay at the cortex.

Acknowledgment

The writer would like to record his thanks to Professor J. J. C. Buckley and to Dr. P. L. LeRoux for their valuable assistance and criticism during the preparation of this work.

Reference

Gharib, H. M., 1960.—"On the migration route of the infective larvae of *Nippostrongylus brasiliensis*." *J. Helminth.*, **35**, 101–108. (W.L. 11224c).

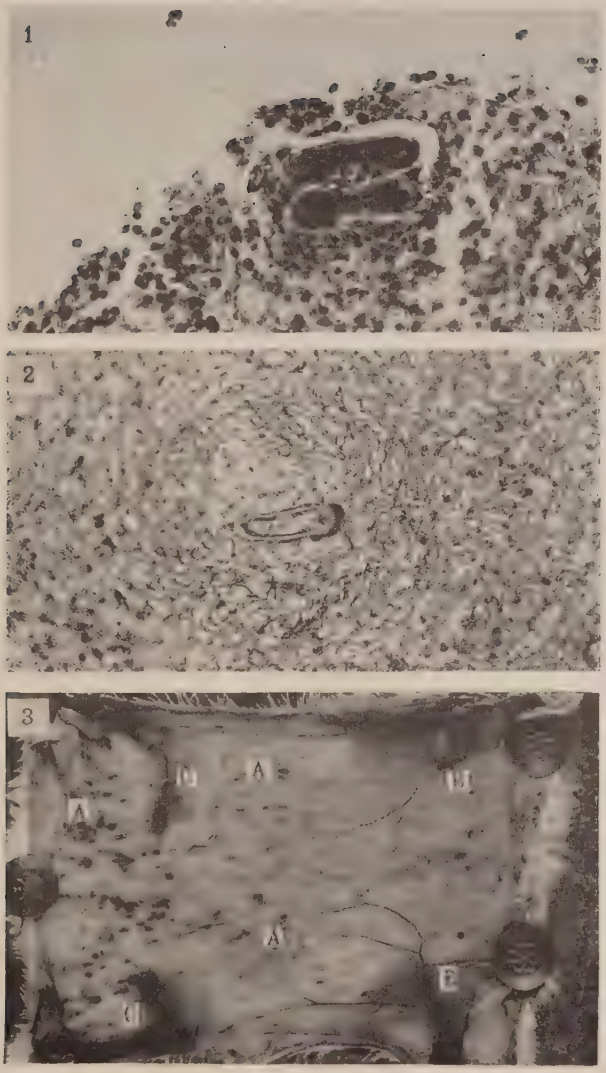


Fig. 1.—Cross section of the precrural lymph gland of a rat showing the larvae of *N. brasiliensis* 12 hours after the exposure of the animal to infection. Note the presence of the parasite at periphery of the lymph gland. (400X). Fig. 2.—Cross section in the precrural lymph of a guineapig showing a marked reaction around the larvae of *N. brasiliensis* 48 hours after exposure in that abnormal host. (200X). Fig. 3.—Under surface of the skin of a mouse (18 hours after exposure) showing marked reaction around : (a) Sites of penetration of the parasite. (b) Axillary lymph glands. (c) Precural lymph glands.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 115–118.

On *Daubaylia elegans* n.sp., in *Planorbis carinatus* Müller

By M. R. Honer* and J. Jansen Jr.

Institute of Veterinary Parasitology and Parasitic Diseases, Utrecht, The Netherlands

During the investigation of parasites in freshwater snails in The Netherlands by one of the present authors (M.R.H.), several specimens (about 30) were found of a species of nematode in the snail *Planorbis carinatus*. On further examination, the nematodes proved to belong to the genus *Daubaylia* Chitwood and Chitwood, 1934, and to be a new species of this genus.

Previously described members of this genus are *Daubaylia potomaca* Chitwood and Chitwood, 1934, *D. dewiti* Schuurmans-Stekhoven 1956 and a species originally described by Baylis and Daubney (1922) as *Cephalobus seistanensis*, but later removed to the genus *Daubaylia* as *Daubaylia seistanensis* by Chitwood and Chitwood (1934).

The new representative of this genus, for which the name *Daubaylia elegans* n.sp., is proposed, may be distinguished from the previously described species in the following tables of comparative measurements.

As will be seen from Table I, the main differences between *D. elegans* n.sp., and the previously described species are the ratio length to breadth and the tail length, which features are brought out in the de Man ratios for the species in Table II.

Other measurements. Maximum width of the body, 0·023—0·026 mm.; width of the anterior end of the body, 0·005–0·006 mm.; width at the level of the annulus, 0·016–0·017 mm.; width of the body at the level of the end of the oesophagus, 0·018 mm. and at the level of the anus, 0·015–0·016 mm.

The measurements of the three divisions of the oesophagus are as follows : corpus, 0·008 mm.; isthmus, 0·005–0·006 mm.; and the pseudobulb, 0·011–0·013 mm.

In no case were eggs observed *in utero* and it is disputable whether eggs are in fact produced (as in most nematodes) because so many larval stages were found with the adults. In addition, the structure of the uterus and its small size suggests that eggs are not produced in the normal manner.

* Part of work carried out during tenure of Research Scholarship from the Ministry of Education, Arts and Sciences, The Netherlands.

With regard to the male specimens found, a similar table of comparative measurements can be made, and are given in Table III.

Although there are differences in the de Man ratios, they are not so striking as in the case of the females, as can be seen in Table IV.

Daubaylia elegans n.sp.

Host : *Planorbis carinatus*

Location : In mantle cavity and body wall.

Locality : Maarssen, The Netherlands.

Type specimens : Institute of Veterinary Parasitology and Parasitic Diseases, State University, Utrecht.

Diagnosis. Characters of the genus *Daubaylia*. Measurements as given above. Females characterized by cuticular knob-like process 0·016–0·028 mm. from the posterior extremity, (see Figure 1). Males distinguished from other species by small size of the spicules and the gubernaculum. The number and distribution of the genital papillae are identical with those described and figured by Schuurmans-Stekhoven (1956) (See Figure 2).

TABLE I

FEMALES

Measurement	*D. potomaca*	*D. seistanensis*	*D. dewiti*	*D. elegans* n.sp.
Body length	1743–1840	1300–1430	2128	1853–2071
Body width	30–40	35	28	23–26
Length, oesophagus	213–239	200–270	300	238–258
Annulus, from anterior end.	125–180		273	135–160
Excretory pore, from anterior end	150–172			155–175
Tail length	100–106	80	68	110–133
Vulva, from anterior end	1000–1060	815	1260	1028–1234
Gonad, from anterior end	650–740		700	825–838
Gonad, from vulva	340–450			203–225

All measurements in μ

TABLE II

De Man Ratios — Females

	D. potomaca	*D. seistanensis*	*D. dewiti*	*D. elegans* n.sp.
α		38–40·85	76	75–86
β	7–8·5	4·75–5·3	7·1	7·6–8·6
γ		17·8	31·3	14·6–17·8
V			54·2	54·9–62

TABLE III

Males

Measurement	*D. potomaca*	*D. seistanensis*	*D. dewiti*	*D. elegans.* n.sp.
Body length	1520–1820	950–1080	1252–1516	1169–1417
Body width	24–30	25	24	21–28
Length, oesophagus	187–212	200–270	142–220	185–200
Annulus, from anterior end	116–125		120–136	108–118
Excretory pore,	160–175			118–135
Length Tail	52–56	50	48–80	43–68
Spicule length	28–29	26	28	22–25
Length, Gubernaculum	16	15	19	12–15
Gonad, from anterior end	640–660		620–740	521–625

All measurements in μ

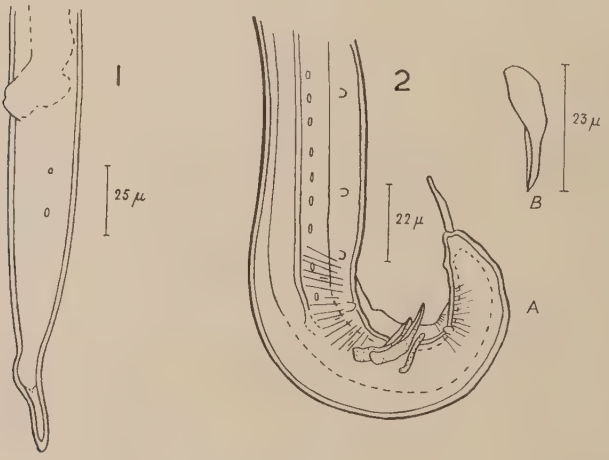
TABLE IV

De Man Ratios — Males

	D. potomaca	*D. seistanensis*	*D. dewiti*	*D. elegans*, n.sp.
α		38–40·85	58–63·1	47–58·1
β	7–8·5	4·75–5·3	6·3–7	7–7·5
γ		19	15·7—18·3	20·5–28·3

Summary

Daubaylia elegans n.sp., is described from *Planorbis carinatus* in the Netherlands. Tables are given of the comparative measurements of the other members of the genus. The main differences between *D. elegans* n.sp., and these other species are the ratio length to breadth, the length of the tail, the presence of a cuticular knob on the tail of the female and the relative size of the male genital armature.



Daubaylia elegans n.sp.

Fig. 1.—Tail of female to show characteristic knob-like projection terminally and the relative slender build of the body.

Fig. 2.—A=Tail of male, showing typical curved terminal part and its spine-like projection and the distribution of the papillae. B=Enlarged drawing of one spicule.

References

Baylis, H. A. and Daubney, R., 1922.—"Report on the parasitic nematodes in the collection of the Zoological Survey of India." *Mem. Indian Mus.*, **7**, 263–347. (W.L. 13401).

Chitwood, B. G. and Chitwood, M. B., 1934.—"*Daubaylia potomaca* n.sp., a nematode parasite of snails, with a note on other nemas associated with molluscs." *Proc. helminth. Soc. Wash.*, **1**, 8–9. (W.L. 16747a).

Schuurmans-Stekhoven, J. H., 1956.—"*Daubaylia dewiti* n.sp., nematodo parasito de *Planorbis contortus* (L.)" *Rev. iber. Parasit.*, **16**, 277–282. (W.L. 18858c).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 119–132.

The Life History of *Echinostoma londonensis* n.sp., the Adult of *Cercaria londonensis* Khan, 1960*

By D. Khan, Ph.D.

From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

Cercaria londonensis Khan, 1960 was reported to be parasitizing a high percentage of *Planorbis corneus* (Linn.) in two ponds in greater London. Experimentally this cercaria penetrated several species of snails and the cysts when fed to pigeons developed into a species of *Echinostoma* Rud., 1809.

The life cycle was repeated in the laboratory using laboratory-bred intermediate and final hosts. The descriptions of the different stages in the life history, other than the redia and cercaria which have already been described (Khan, 1960) are given here.

Egg

(Fig. 1)

The eggs are oval, operculate and brownish-yellow in colour. The eggs collected from faeces measure 0·1–0·12 mm. long and 0·06–0·075 mm. broad and are unembryonated when laid.

Miracidium

(Figs. 2, 3, 4 and 5)

The eggs were collected from experimentally infected pigeons, concentrated and allowed to incubate in distilled water at the room temperature. The miracidium was found to be fully formed in four weeks. At this stage the miracidium exhibits slow movements of contraction and extension after long intervals. The essential structures such as eyespots, flame cells, cilia and to some extent the epidermal plates may easily be seen through the egg shell. As the miracidium is longer than the egg shell, it lies with a bend on the ventral side and occupies more than half the space inside the shell. The rest of the space is occupied by two refractile globules. The apical papilla is almost always facing the opercular end.

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

The emergence of the miracidia commences after five weeks. All the miracidia do not hatch in one day and the hatching continues for about one week. The emergence was seen to be particularly facilitated by change of water and exposure to strong light. In the absence of these influences very few miracidia were found to hatch.

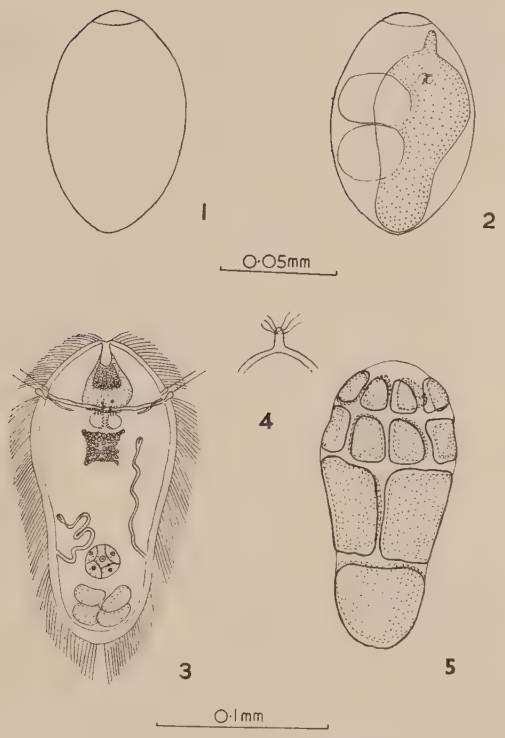
When the miracidium is ready to hatch, it becomes very active and strikes against the operculum vigorously with the apical papilla. The body contracts and expands quickly and the cilia start beating very actively.

The liberated miracidia are very active and swim in all directions. Their movements may be temporarily slowed down on striking against a solid object or on reaching the surface of the water. When swimming at a lower speed the body is seen to be rotating on its long axis along with forward movement. In a Petri dish, although they are found in all parts and depths of the water, more of them are always found near the periphery, slightly below the surface and nearest to the light source.

To determine whether the miracidia show any response to light, one half of the Petri dish was placed in comparative darkness, while the other half was exposed to strong light. It was noticed that a much larger proportion of the miracidia were always found in the lighted half of the container and only a few were seen in the dark area.

The miracidium is elongated with the maximum girth at about one third of its length from the anterior end. The living miracidia measure from 0·13–0·14 mm. in length, 0·071–0·08 mm. in breadth. The body is covered with long cilia borne on nucleated epidermal plates. The epidermal plates (Fig. 5) are arranged in 4 tiers. The first tier has six, roughly triangular plates, of which two are dorsal, two lateral and two ventral in position. The epidermal plates of the first tier measure 0·018–0·023 mm. long with a maximum breadth of 0·013–0·016 mm. The second tier also has six epidermal plates of which two are dorsal, two lateral and two ventral in position. The epidermal plates of the second tier are squarish in shape and measure 0·024–0·027 mm. long and 0·018–0·022 mm. broad.

The third tier has four rectangular epidermal plates of which two are dorso-lateral in position and two are ventro-lateral. The epidermal plates of the third tier measure 0·05–0·07 mm. long and 0·03–0·037 mm. broad. There are only two epidermal plates in the last tier. One of these is dorsal in position and the other ventral, measuring 0·03–0·04 × 0·04–0·05 mm. The arrangement of the epidermal plates may be expressed as 6 : 6 : 4 : 2.



Echinostoma londonensis n.sp.

Fig. 1.—Egg capsule. Fig. 2.—Egg, with a fully developed miracidium inside. Fig. 3.—Miracidium, general structure. Fig. 4.—Anterior end of the miracidium, showing the apical papilla. Fig. 5.—Epidermal plates of the miracidium (dorsal view).

The distance between the first and the second tiers of epidermal plates is greater than between other tiers. The cilia, which beat from the anterior posteriorly are about the same size on all plates. Very often the cilia at the anterior and posterior ends seem to be longer than at the other parts of the body. This is because at these places the cilia are not very sharply bent backwards and their full length may be seen.

At the anterior end of the body is a retractile apical papilla (Fig. 4). This papilla can be completely withdrawn and may remain

so for a long time. In several cases when a miracidium was being studied, this organ was found to be completely retracted. Slightly behind the tip of the apical papilla is a circle of long hair-like structures, about six to eight in number and longer than the body cilia but inactive ; they might be sensory in function. No mouth opening could be seen.

Between the first and second tier of the epidermal plates is a collar-like fold of the body. Laterally between the first and the second tier of the epidermal plates on each side is a pair of small processes, the lateral processes. Each of them has a narrow stalk and a swollen apical part. The apical part of one of these lateral processes on each side is broader than the other. Each of them bears a long hair-like process, longer and stiffer than the body cilia and inactive. A very fine duct was seen to proceed from these lateral processes towards the central nerve mass, but its connection with any internal structure could not be established. Very often, particularly when the miracidium is about to die, a small droplet was seen exuding from the tip of these processes.

Just behind the apical papilla are four glands, the penetration glands, with very coarsely granular contents and clear nuclei. The walls of the penetration gland cells are poorly developed and are difficult to see. They may often appear as a single flask-shaped structure with four nuclei at the posterior end. Their contents seem to extend, probably into their duct, up to the tip of the apical papilla.

Extending from behind the apical papilla, to the level of the eyespots, lying beneath the penetration glands, is a broad, saccular organ, the so-called "primitive gut". The contents of this organ are finely granular and homogeneous and at the posterior end there are four large nuclei. The walls of this organ do not exhibit a cellular nature and there are no appendages to this organ. In miracidia which are about to die under the pressure of a cover glass a small drop of clear liquid was seen to ooze out of the tip of the apical papilla. This liquid was, however, quite unlike the contents of the penetration glands or of the primitive gut. The central nervous system is represented by a quadrangular mass of nervous tissue with several superficial nuclei, possibly of the nerve cells. On the anterior and dorsal side of the central nerve mass is a pair of prominent eyespots. The eyespots consist of a pair of crescents of dark granules, with a clear lens on each concave side. These eyespots may touch each other mesially or may even overlap, but are never fused into a single mass.

There is one pair of asymmetrically placed flame cells. The anterior, on the right side is situated at the level of the eyespots.

The posterior or the left flame cell is nearly in the middle of the third tier of epidermal plates. The right flame cell is ventral in position while the left is dorsal. The excretory duct of each flame cell opens independently between the third and fourth tier of epidermal plates on the corresponding side.

The germinal mass is represented by a compact mass of nucleated cells located between the third and the fourth tiers of epidermal plates. At the posterior end of the body, there are four cells more prominent and larger than the other cells in the body. Their nature is not known (Fig. 3).

Discussion. The number and the arrangement of the epidermal plates in the present miracidium is the same as described for *Hypoderaeum conoideum* (Mathias, 1925); *Echinoparyphium recurvatum* (Rasin, 1933); *E. flexum* (Najarian, 1954); *Echinostoma revolutum* (Beaver, 1937), but differs from that described for *Parorchis acanthus* (Rees, 1940) and *Euparyphium ilocanum* (Tubangui and Pasco, 1933). This variation among the members of the same family upholds the view of Lynch (1933) and Dobrovolny (1939) and that the number and the arrangement of the epidermal plates is not a reliable index of the family relationships as suggested by several workers (Stunkard, 1923; LaRue, 1926; Price, 1931; Hunter and Hunter, 1935; Bennet, 1936; Beaver, 1937 and Tang, 1941).

Willmott (1952) has denied that the lateral processes are present in the miracidium of *Paramphistomum hiberniae* and regards them as nothing but cytoplasmic extrusions, which appear specially in the moribund miracidia. In the present miracidium, however, these structures were clearly present and are also described for the miracidia of *P. acanthus* (Rees, 1940) and *E. flexum* (Najarian, 1954). Tubangui and Pasco (1933), however, describe only one pair of processes for *E. ilocanum*. The structure of the lateral processes in the miracidium under study is more like the one described for several strigeid miracidia, in having long hair-like structures attached to each of them. Although a small duct was seen to proceed inwards from the lateral processes on each side, its definite connection with any internal structure could not be established, as has been shown by Faust and Meleney (1924), Hunter and Hunter (1935) and Sinha and Srivastava (1960), in some strigeid miracidia.

The so-called "primitive gut" has been regarded as a functional gut full of granular digested material derived from vitelline cells of the egg; as a rudimentary non-functional gut; as a glandular organ and as a rudimentary gut which affects the penetration of the miracidium into the snail, through external digestion of the host-tissue.

I was unable to find a true mouth opening as shown by Barlow (1925), Sinitsin (1931) and Rees (1940). Price (1931) and Tubangui and Pasco (1933) were also unable to see a mouth opening in other miracidia.

The pigment of the eyespots was never seen to be fused into a single mass as shown in *E. revolutum* (Johnson, 1920); *E. murium* (Tubangui, 1932); *E. ilocanum* (Tubangui and Pasco, 1933) and *E. flexum* (Najarian, 1954).

Penetration into Snail

When placed in a watch glass along with a prospective snail host, the miracidium does not exhibit any chemotactic reaction, nor does it swim directly towards the snail. Any contact with the snail is purely accidental, which may occur during the course of its normal swimming. Even after touching such parts of the snail which might not be suitable for penetration, it usually swims away from it after a few seconds of probing with its apical papilla. Very often a miracidium may get entangled in the slime secreted by a snail. In such event its movements are very much retarded, but a slow forward movement through the slime may continue for some time.

The snail reacts violently to the penetration of a miracidium which causes it to withdraw itself into the shell and to secrete a thick coat of slime. This secretion might be serving as a mechanical barrier against the entry of any more miracidia.

In the present study only young *P. corneus* were used for the above experiments. Most of the snails which had been exposed to the miracidia were found to die after six to seven days.

The actual penetration of the miracidium into the snail was not observed.

Redia and cercaria have been described previously (Khan, 1960).

Cyst

The structure and the size of the cyst has already been described (Khan, 1960). It was not possible to break the cyst without causing considerable damage to the metacercaria.

Although echinostome cercariae have been reported to encyst in the open and also in a wide variety of hosts, only *Lymnaea stagnalis*, *L. pereger* and *P. corneus* were used as second intermediate hosts for the present cercaria, which was never found to encyst in the open.

Whenever all these species of snails were exposed to the cercariae in the same container it was always found that the largest number of cercariae encysted in *L. pereger* and the smallest number in *P. corneus*. It is suggested that when the opportunity presents itself, the echinostome cercariae show some host preference as was also noted by Beaver (1937) in *E. revolutum*.

It was also noted that when snails harbouring the rediae and those free of any infection are kept in the same tank for some weeks, much smaller numbers of cysts are found in the snails harbouring the rediae than in other snails. Also, when snails were exposed to cercariae in a small container, several hours (at least 8) passed before any noticeable number of cercariae were found to have penetrated into the snails. These two observations suggest that *C. londonensis* definitely leaves the first intermediate host and requires a free swimming life before it can penetrate into the same snail or another snail before encysting.

Localization of the cysts on a particular organ of the second intermediate host has also been noted for several other echinostome cercariae (Sewell, 1922; Mathias, 1925; Brown, 1926; McCoy, 1929; Wesenberg-Lund, 1934; Ono, 1935 and Johnston and Angel, 1941).

No growth in the size of the cyst with increasing age was noticed in the present case as stated by Krull (1935) in *E. coalitum*.

FEEDING EXPERIMENTS

Experiment No. 1

			Examination of faeces									
		Days after feeding—	1	2	3	4	5	6	7	8	9	Results of autopsy
Animal	No. of cysts fed	Age of cysts										
Rat 1	200	4 weeks	—	—	—	—	—	—	—	—	—	Nil
Rat 2	100	4 weeks	—	—	—	—	—	—	—	—	—	Nil
Rat 3	100	4 weeks	—	—	—	—	—	—	—	—	—	Nil
Duckling 1	200	4 weeks	—	—	—	—	—	—	—	—	—	Nil
Duckling 2	100	4 weeks	—	—	—	—	—	—	—	—	—	Nil
Pigeon 1	100	4 weeks	—	—	—	—	—	—	—	+	+	10 adults
Pigeon 2	200	4 weeks	—	—	—	—	—	—	—	+	+	20 adults
Pigeon 3	500	4 weeks	—	—	—	—	—	—	died of acute enteritis			
Pigeon 4	1000	4 weeks	—	—	—	—	—	—	died of acute enteritis			

Experiment No. 2

Animal	No. of cysts fed	Age of cysts	Examination of faeces: Days after feeding— 1	2	3	4	5	6	7	8	9	Results of autopsy
Pigeon 5	100	1 day	—	—	—	—	—	—	—	—	—	Nil
Pigeon 6	200	1 day	—	—	—	—	—	—	—	—	—	Nil
Pigeon 7	100	2 days	—	—	—	—	—	—	—	—	—	Nil
Pigeon 8	200	2 days	—	—	—	—	—	—	—	—	—	Nil
Pigeon 9	100	3 days	—	—	—	—	—	—	—	—	—	Nil
Pigeon 10	200	3 days	—	—	—	—	—	—	—	—	—	Nil
Pigeon 11	100	4 days	—	—	—	—	—	—	—	+	+	15 adults
Pigeon 12	200	5 days	—	—	—	—	—	—	—	+	+	23 adults
Pigeon 13	100	6 months	—	—	—	—	—	—	—	+	+	18 adults

Experiment No. 3

Animal	No. of cysts fed	Age of cysts	Examination of faeces: Days after feeding 9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Pigeon 14	100	2 weeks	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—
Pigeon 15	100	2 weeks	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Pigeon 16	100	2 weeks	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—
Pigeon 17	100	2 weeks	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—
Pigeon 18	100	2 weeks	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—

+ = eggs present in faeces
— = eggs absent in faeces

Conclusions from the feeding experiments

The following conclusions may be drawn from the above experiments.

1. Rats and ducklings do not serve as final hosts for this trematode. Pigeons are the suitable experimental hosts.
2. About 20 per cent of the cysts develop into adults.
3. The cysts which are less than four days old are not infective.
4. The trematodes attain maturity in eight days after being fed to the final host.
5. In heavy infections the trematodes cause acute enteritis and kill the host.
6. After attaining maturity the trematodes remain for seven to thirteen days in the host after which they are passed out.

The adult trematodes are always found in the large intestine of the host. In no pigeon which was dissected was any adult found in the small intestine.

ADULT

Echinostoma londonensis n.sp.

(Figs. 6—9)

The general shape of the body is elongated, dorso-ventrally flattened, with the greatest width usually in the uterine region, but sometimes in the region of the ventral sucker.

There is a well-developed collar at the anterior end. The collar is continuous over the dorsal side and bears 37 spines (Fig. 9). Five of these collar spines on each side constitute a corner group, with three oral in position and two more deeply set and aboral. The outer aboral corner spine is always larger than the rest. There are 4 lateral unalternating spines on each side. The 19 dorsal spines are arranged in two rows of alternating spines, the oral series with 10 spines and the aboral series with 9. The rows of dorsal spines are continuous over the dorsal side. The aboral dorsal spines are continuous with the lateral spines. In properly fixed specimens, which were fixed soon after the dissection of the host, the number and the arrangement of spines was found to be constant.

The body spination extends from just behind the collar to the level of the ventral sucker on the dorsal side and to the posterior testis or sometimes slightly beyond on the ventral and the lateral sides.

The oral sucker is sub-terminal, with a uniform edge to its aperture and is followed by a very short prepharynx, which is usually completely hidden in whole mounts. The muscular pharynx is well developed and only slightly smaller than the oral sucker. The pharynx is usually longer than broad. The oesophagus divides just in front of the ventral sucker and the caeca extend nearly to the posterior end of the body. The ventral sucker is about one sixth to one eighth of the body length from the anterior end, and about two and a half times the size of the oral sucker. The edge of its aperture is wavy.

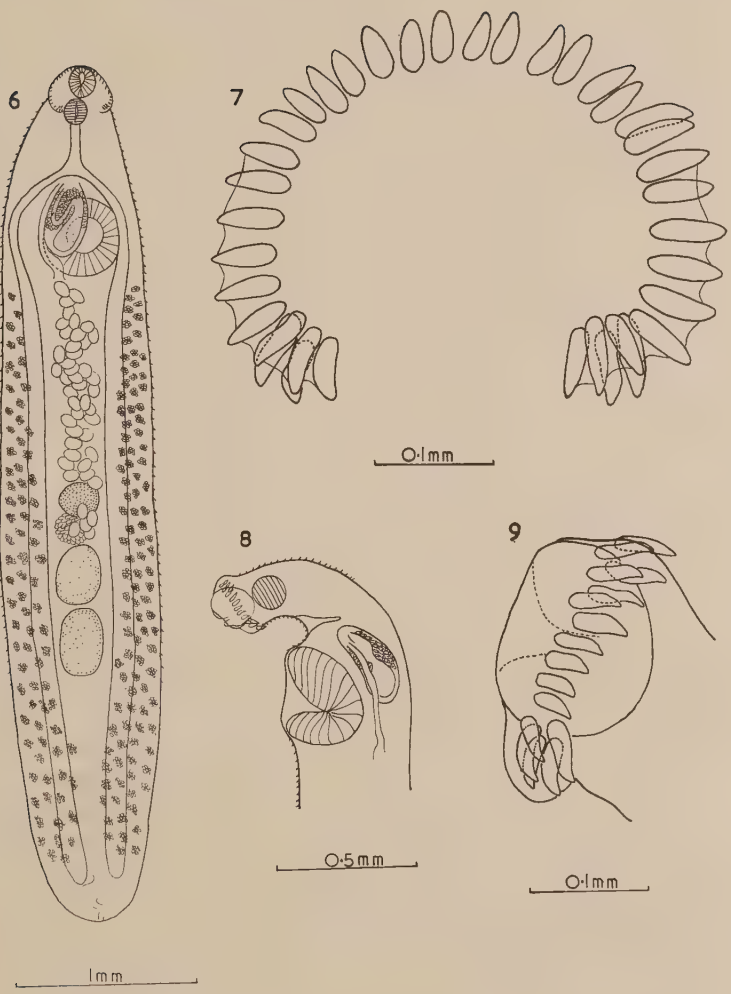
The testes are tandem and nearly spherical to ovoid in shape in specimens fixed without any pressure. In specimens fixed under pressure, they invariably become more or less lobed. They are located in the posterior half of the middle third of the body. The anterior testis is somewhat smaller than the posterior . The pyriform cirrus sac (Fig. 8) is muscular and always lies dorsal to the ventral sucker in specimens fixed without any pressure, but in those fixed

under pressure it comes to lie to the right side of the ventral sucker. In size the cirrus sac is as long as or slightly smaller than the ventral sucker but less broad. The seminal vesicle is well developed and coiled. The pars prostatica is voluminous and the muscular, unspined cirrus when fully protruded may be even longer than the cirrus pouch.

The ovary is situated just behind the equator and is spherical in shape. The diffuse shell gland is well developed and nearly the same size as the ovary. The uterus has 8 to 10 coils up to the ventral sucker, after which it runs alongside the cirrus sac and opens into a common genital aperture, which is immediately post-bifurcal. A proper common genital atrium is not present. The vitellaria are in the shape of small rosette-like clusters, extending from just behind the ventral sucker to near the posterior end of the body. In front of the anterior testis the vitellaria are extracaecal. Behind the testicular region, they extend into the intercaecal space, but never meet in the middle.

Measurements (in mm.) of 9-day-old Adults

Body length	4·05–5·4
Body breadth	0·405–0·93
Collar across	0·3–0·375
Oral sucker	0·15–0·195 × 0·135–0·18
Prepharynx	0·015–0·048
Pharynx	0·12–0·165 × 0·12–0·135
Oesophagus	0·225–0·345
Ventral sucker	0·36–0·51 × 0·39–0·5
Cirrus sac	0·295–0·42 × 0·18–0·27
Ovary	0·12–0·21 × 0·165–0·255
Anterior testis	0·195–0·33 × 0·195–0·285
Posterior testis	0·24–0·375 × 0·21–0·27
Corner spines	
Inner aboral	0·040–0·046 × 0·013–0·016
Middle aboral	0·043–0·050 × 0·013–0·016
Outer aboral	0·043–0·046 × 0·013–0·016
Inner oral	0·040–0·046 × 0·016
Outer oral	0·050–0·060 × 0·016–0·023
Lateral spines	0·046–0·053 × 0·013–0·016
Dorsal spines	
Oral	0·05–0·063 × 0·016–0·020
Aboral	0·046–0·056 × 0·016–0·020



Echinostoma londonensis n.sp.

Fig. 6.—General structure of the adult (dorsal view). Fig. 7.—Collar spination. Fig. 8.—Anterior end of the adult (lateral view). Fig. 9.—Collar spination from lateral side.

Comparison of *Echinostoma londonensis* n.sp. With Related Species

The species of the genus *Echinostoma* Rud., 1809 exhibit a remarkable morphological uniformity. In comparing the present species, particular emphasis has been placed on such characters as the number and the arrangement of the collar spines and the proportion between the two suckers as suggested by Beaver (1937), on the size of the collar spines which has been used by Yamaguti (1933, '34) and Bonne, Bras and Joe (1948) and on the differences in the larval stages.

After excluding the species which have been regarded as synonyms of *Echinostoma revolutum* (Froel., 1802) Dietz, 1909, 7 species remain to be compared with the trematode under study.

E. ralli Yamaguti, 1934 and *E. goldi* Oschmarin, 1956, have a group of four and six corner spines on each side respectively and also differ from the present species in having a different proportion between their two suckers. Similarly, *E.* sp. Sadovskaja, 1952 has a different proportion between the oral and the ventral sucker. The arrangement of the collar spines in this species has not been described in detail, but the figure shows a group of 3 corner spines on one side and only 1 on the other, an arrangement quite different from the present species.

E. revolutum tenuicollis Baschkirova, 1941; *E. lindoensis* Sandground and Bonne, 1940; *E. robustum* Yamaguti, 1935 and *E. stromi* Baschkirova, 1946 all have larger collar spines than in the present species. *E. revolutum tenuicollis*, *E. lindoensis* and *E. robustum* also have a different proportion between the two suckers, while in *E. stromi* the pharynx is less than half the size of the oral sucker. *E. lindoensis* can also be separated on the differences between the cercarial stage and that of the present species (Khan, 1960).

E. revolutum differs from the present species in having six lateral spines on each side, in having a different proportion between the two suckers and in the larger size of the collar spines. Cercaria of *E. revolutum* can also be easily distinguished from *Cercaria londonensis*. Furthermore the present species failed to develop in rats while *E. revolutum* readily develops in them.

On the basis of above comparison, the present species is regarded as new and the name *Echinostoma londonensis* is proposed for it.

Summary

Cercaria londonensis Khan, 1960 is shown to develop into a new species of *Echinostoma*. The stages in the life history are described.

ACKNOWLEDGMENTS

I am grateful to Prof. J. J. C. Buckley, under whose kind guidance this work was carried out. My thanks are also due to Dr. P. L. LeRoux of the London School of Hygiene and Tropical Medicine for his help and advice.

REFERENCES

BARLOW, C. H., 1925.—"The life cycle of human intestinal fluke, *Fasciolopsis buski* (Lankester)". *Monogr. Ser. Amer. J. Hyg.*, **4,** 1–98. (W.L. 14428b).

BASCHKIROVA, E. I., 1941.—(In Skrjabin, 1956 : Trematodes of Animals and man, **12**).

————, 1946.—"Two new echinostomids of birds in Azerbaijan". *Collected papers on helminthology dedicated by his pupils to A. K. I. Skrjabin in his 40th year of scientific teaching and administrative achievement.* pp. 42–46.

BEAVER, P. C., 1937.—"Experimental studies on *Echinostoma revolutum* (Froel.) a fluke from birds and mammals." *Illinois biol. Monogr.*, **15,** 1–96. (W.L. 9822).

BENNET, H. J., 1936.—"The life history of *Cotylophoron cotylophorum*, a trematode of ruminants." *Ibid.*, **14,** 1–119.

BONNE, C., BRAS, G. and JOE, K. L., 1948.—"Five human echinostomes in Malayan Archipelago." *Overged. Medisch Haandblad.* No. **23**, 456–465.

BROWN, F. J., 1926.—"Some British freshwater larval trematodes with contributions to their life histories." *Parasitology*, **18,** 21–34. (W.L. 16035).

DIETZ, E., 1909.—"Die Echinostomiden der Vögel." *Zool. Anz.*, **34,** 180–192. (W.L. 23833).

DOBROVOLNY, C. G., 1939.—"Life history of *Plagioporus sinitsini* Mueller, and the embryology of new cotylocercous cercariae (Trematoda)." *Trans. Amer. Micr. Soc.*, **58,** 121–155. (W.L. 21400).

FAUST, E. C. and MELENEY, H. E., 1924.—"Studies on *Schistosomiasis japonica.*" *Monogr. Ser. Amer. J. Hyg.*, No. **3**, 1–339. (W.L. 14428b).

HUNTER, W. S. and HUNTER, G. W., 1935.—"Studies on *Clinostomum.* II The miracidium of *C. marginatum* (Rud.)." *J. Parasit.*, **21,** 186–189. (W.L. 11428).

JOHNSON, J. C., 1920.—"The life cycle of *Echinostoma revolutum* (Froel. 1802)." *Univ. Calif. Publ. Zool.*, **19,** 335–388. (W.L. 22084).

JOHNSTON, T. H. and ANGEL, L. M., 1941.—"A life history of *Echinostoma revolutum* in South Australia." *Trans. roy. Soc. S. Aust.*, **65,** 317–322. (W.L. 21654).

KHAN, D., 1960.—"Studies on larval trematodes infecting freshwater snails in London and some adjoining areas. Part I. Echinostome cercariae." *J. Helminth.*, **34,** 277–304. (W.L. 11224c).

KRULL, W. H., 1935.—"A note on the life history of *Echinostoma coalitum* Barker and Beaver, 1915 (Trematoda : Echinostomatidae)." *Proc. helm. Soc. Wash.*, **2,** 76. (W.L. 16747a).

LARUE, G. R., 1926.—"Studies on the trematode family Strigeidae (Holostomidae) No. III. Relationships." *Trans. Amer. micr. Soc.*, **45,** 265–281. (W.L. 21400).

LYNCH, J. E., 1933.—"The miracidium of *Heronimus chelydrae* McCallum." *Quart. J. micr. Sci.*, **76,** 13–33. (W.L. 17510).

MATHIAS, P., 1925.—"Recherches expérimentales sur la cycle évolutif de quelques Trematodes." *Bull. biol.*, **49,** 1–123. (W.L. 3919).

McCOY, O. R., 1929.—"Notes on cercariae from Missouri." *J. Parasit.*, **15,** 199–208. (W.L. 11428).

NAJARIAN, H. H., 1954.—"Developmental stages in the life cycle o *Echinoparyphium flexum* (Linton, 1892) Dietz, 1910 (Trematoda : Echinostomidae)." *J. Morph.*, **94,** 165–197. (W.L. 11373).

ONO, S., 1935.—"Studies on the trematodes invading *Lymnaea* snails as first intermediate hosts, found in the vicinity of Mukden. II. On the encystation and development of Echinostomidae." *J. Jap. Soc. vet. Sci.*, **14,** 232–246. (W.L. 11278).

OSCHMARIN, P. G., 1946.—(In Skrjabin, 1956 : Trematodes of animals and man, **12.**)

PRICE, H. F., 1931.—"The life history of *Schistosoma douthitti* Cort". *Amer. J. Hyg.*, **13,** 685–727. (W.L. 600a).

RASIN, K., 1933.—"*Echinopharyphium recurvatum* (Linstow, 1873) und seine Entwicklung." *Biol. Spis. vys. Sk. Zverolek.*, **12,** 1–104. (W.L. 2977a).

REES, F. G., 1940.—"Studies on the germ cell cycle of the digenetic trematode, *Parorchis acanthus* Nicoll. Part II. Structure of miracidium and germinal development in the larval stages." *Parasitology*, **32,** 372–391. (W.L. 16035).

SADOVSKAJA, 1952.—(In Skrjabin, 1956 : Trematodes of animals and man, **12.**)

SANDGROUND, J. H. and BONNE, C., 1940.—"*Echinostoma lindoensis*, a new parasite of man in the Celebes with an account of its life history and epidemiology." *Amer. J. trop. Med.*, **20,** 511–535. (W.L. 626).

SINHA, P. K. and SRIVASTAVA, H. D., 1960.—"Studies on *Schistosoma incognitum* Chandler, 1926. II. On the life history of the blood fluke." *J. Parasit.*, **46,** 629–641. (W.L. 11428).

SINITSIN, D., 1931.—"Studien über die Phylogenie der Trematoden." *Z. wiss. Zool.*, **138,** 409–456. (W.L. 23635).

STUNKARD, H., 1923.—"Studies on North American blood flukes." *Bull. Amer. Mus. nat. Hist.*, **48,** 165–221. (W.L. 3768).

TANG, C. C., 1941.—"Morphology and life history of *Prosostephanus industrius* (Tubangui, 1922) Lutz, 1935 (Trematoda : Cyathocotylidae)." *Peking nat. Hist. Bull.*, **15,** 299–316. (W.L. 16087b).

TUBANGUI, M. A., 1932.—"Observations on life of *Echinoparyphium murium* Tubangui 1931 and *Echinostoma revolutum* (Froel. 1802) (Trematoda)." *Philipp. J. Sci.*, **47,** 497–513. (W.L. 16189).

TUBANGUI, M. A. and PASCO, A. M., 1933.—"The life history of human intestinal fluke, *Euparyphium ilocanum* (Garrison, 1908)." *Ibid*, **51.** (W.L. 16189).

WESENBERG-LUND, C., 1934.—"Contributions to the development of the Trematode Digenea. II. The biology of the freshwater cercariae in Danish freshwaters." *K. danske. vidensk. Selsk.*, **5,** 1–223. (W.L. 11861).

WILLMOTT, S., 1952.—"The development and morphology of the miracidium of *Paramphistomum hiberniae* Willmott, 1950." *J. Helminth.*, **26,** 123–132. (W.L. 11224c).

YAMAGUTI, S., 1933.—"Studies on the helminth fauna of Japan. Part I. Trematodes of birds, reptiles and mammals." *Jap. J. Zool.*, **5,** 1–134. (W.L. 10881w).

————, 1934.—"Studies on helminth fauna of Japan. Part III. Avian trematodes. II." *Ibid.*, **5,** 129–210. (W.L. 10881w).

————, 1935.—"Studies on the helminth fauna of Japan. Part V. Trematodes of birds. III." *Ibid.*, **6,** 159–182. (W.L. 10881w).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961 pp 133-142.

Studies on Larval Trematodes Infecting Freshwater Snails in London (U.K.) and Some Adjoining Areas. Part III. "Lophocercous" Cercariae.*

By D. Khan, Ph.D.
From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

During 1958 and 1959 a survey of the freshwater larval trematodes was carried out in London and some parts of Kent, Essex and Middlesex. Two "Lophocercous" cercariae were found. These cercariae were studied in both living and fixed conditions. For fixation 10 per cent formal-saline (70°–80°C.) was used. For measurement twenty specimens were used in each case.

The group "Lophocercous" cercariae was erected by Lühe (1909) for *C. cristata* La Val. 1855 and *C. microcristata* Ercol. 1881, both of which had a dorsal finfold on the body. He regarded this as a completely separate group of cercariae. Sewell (1922) in accordance with his scheme to divide all the cercariae into two categories on the sole basis of presence or absence of a ventral sucker placed the "Lophocercous" cercariae among his "Monostome Cercariae". This view was not accepted by later workers. However, Sewell gave a detailed definition of this group.

Miller (1926) included these forms among his "Apharyngeal Brevifurcate Monostome" division of Furcocercariae.

Odhner (1911) suggested that *C. cristata* would develop into a species of *Sanguinicola* Plehn, 1908. The work of Scheuring (1923) and Ejsmont (1926) has proved this to be true. The last two authors added three more species to the "Lophocercous" cercariae. Dubois (1929) added another species, *C. helvetica XVI* from Neuchâtel. Porter (1938) has described two species from Africa. More recently Johnston and Beckwith (1947) and Fain (1953) have added one species each from Australia and Belgian Congo respectively.

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

According to Dawes (1946) these cercariae are confined to the Old World. Some cercariae, such as *C. spirorchis parvus* Wall, 1940, *C. whitentoni* Croft, 1953 and *C. brevifurca* McCoy 1929, have been described from the United States. All these have a body finfold. Wall (1941) also described another cercaria belonging to the same genus—*C. spirorchis elephantis*—which is without a body finfold. It is clear that the presence of a dorsal body finfold alone is not sufficient to justify a natural group, but, as pointed out by Johnston and Beckwirth (1947), none of these American forms as well as *C. bombayensis* No. 8 Soparker, 1921 and *C. Clinostomum marginatum* as described by Krull (1934) would be included in the group if Sewell's definition is adopted. This would leave in the group "Lophocercous", cercariae which exhibit a remarkable similarity in morphology and wherever the life cycle has been worked out, they have been shown to penetrate directly into cyprinoid fishes and develop into species of *Sanguinicola*, in the blood vascular system.

So far no species of this group has been reported from Britain.

Cercaria kentensis n.sp.

From Keston Common (Kent) twenty *L. pereger* (Müll.) were collected on 31st April 1959. Of these, ten snails were found to be emitting this cercaria. All of the infected snails were small sized and young. An equally high percentage of infection was found among *L. pereger* collected on several occasions during May 1959, from Sand Pits near South Ockendon (Essex). The infection rate fell considerably in snails collected from either of the above places during June (20 per cent) and was rare in snails collected during July (2 per cent). No infected snail was found in August or later in the season. Always small sized snails were found to be infected with this cercaria.

Sporocyst

Infected snails which had been kept in the laboratory for four weeks stopped discharging the cercariae. The digestive glands of these snails on dissection were found to be completely free of any sporocysts or developing cercariae. On the other hand, some of the snails which were still emitting the cercariae, were found to have a large number of cercariae in various stages of development lying free in the tissue of the digestive gland. The snails, collected very early in the season (early May) had very delicate, thin-walled, elongated sac-like sporocysts in the digestive gland of the host. These had

numerous cercariae in a very early stage of development. The sporocysts are so delicate that they rupture with the slightest touch of a dissecting needle. They are firmly embedded in the host tissue and are very difficult to separate. They have no birth pore. The large number of cercariae, as they increase in size, cause the rupture of the sporocysts and are liberated still very immature, into the host tissue, where they complete their development before they are discharged.

Wesenberg-Lund (1934) described the sporocyst of a closely related form, *C. cristata*, which had daughter sporocysts in it ; this was presumably a mother sporocyst. No mother sporocyst was found in the present study. From the above observation and the fact that no large sized snail was ever found to be infected with this parasite it is suggested that the sporocysts, after having liberated their contents, disintegrate and leave the host completely free of infection and probably immune to reinfection.

Complete living sporocysts measured 1·0–1·12 mm. long and 0·15–0·16 broad.

Emergence and Behaviour

The cercariae are shed in the late afternoon and perhaps during the night or early morning also. They are emitted in very large numbers. After emergence they remain well distributed throughout the length of the container and do not show any phototropism. Usually they hang motionless in water and do not swim for a long time if undisturbed. While resting the furca are wide open and the tail shaft is bent to such an extent that the proximal half of the tail stem and the body comes to lie against the distal half of the tail shaft. The tip of the body touches the furca. In this position, in which furca are kept upwards, the portion of the tail stem just behind the body is twisted into a right angle, so that the ventral side of the body comes to lie against the lateral side of the tail stem. This long resting period is followed by a very short period of swimming during which the cercaria darts for a short distance, tail foremost.

Cercaria

The body of the cercaria is very small and laterally compressed. There is a finfold on the mid-dorsal line. This finfold is reduced at the anterior as well as the posterior end, starting from a point at a distance from the anterior organ and finishing a small distance in front of the junction of body with tail. The lateral compression and the finfold causes the cercaria to lie on its side under a cover

glass. The anterior organ is modified to form a protrusible telescopic snout, which is demarcated from the rest of the body. At the anterior tip there are two conical, perhaps hollow processes, which presumably aid in its penetration into the final host. Behind a small bare anterior part there are four circular rows of spines encircling the anterior half of the anterior organ. The rest of the body is without any spines. There is no ventral sucker. In some of the related cercariae a simple rhabdocoele gut has been reported [*C. cristata*; *C. microcristata*; *C. sanguinicola inermis* Scheuring; *C.* species (*B. leechi*) Ejsmont, 1926; *C.* species (*L. stagnalis*) Ejsmont, 1926]. In the present species I have not found any trace of a gut. There are several cells inside the body with granular cytoplasm and nuclei, and these it has been suggested, are penetration gland cells. Six of these occupy an area immediately behind the middle of the body; they are prominent and have six ducts, the penetration gland ducts, opening at the anterior end. Two less prominent glands, with clearer cytoplasm are seen on the antero-dorsal aspect of the six glands just mentioned, with their ducts opening subterminally on the ventral side of the anterior organ. There are some other cells present in the body but their nature or their ducts could not be ascertained.

The excretory bladder is two chambered, with a main excretory duct proceeding forwards from each of them. Three flame cells are present on each side of the body, but their connection with excretory ducts could not be made out. Posteriorly from each chamber of the excretory bladder one caudal excretory duct originates. These ducts proceed throughout the length of the tail shaft, intercoiling at two or three places, but never joining each other. Each of these caudal excretory ducts passes into one of the furca and opens into its cup-like tip. There are no flame cells in the tail.

The genital primordium is represented by a single mass of deeply staining cells lying just in front of the excretory bladder.

The tail shaft is nearly twice the length of the body. There is a core of numerous rounded bodies extending throughout its length. The tail shaft has spines scattered all over its surface and several fine hair-like structures, of which fifteen have been observed on each side. A well developed series of oblique muscle fibres originate at the lateral margin and terminate at the centre of the tail shaft. The furca are short, laterally compressed, blade-like structures. They are provided with a finfold on their dorsal and ventral sides, extending over its entire length and continuous over the tip. Like the tail shaft, the furca are also provided with spines, irregularly scattered over it, but there are no "flagellets".

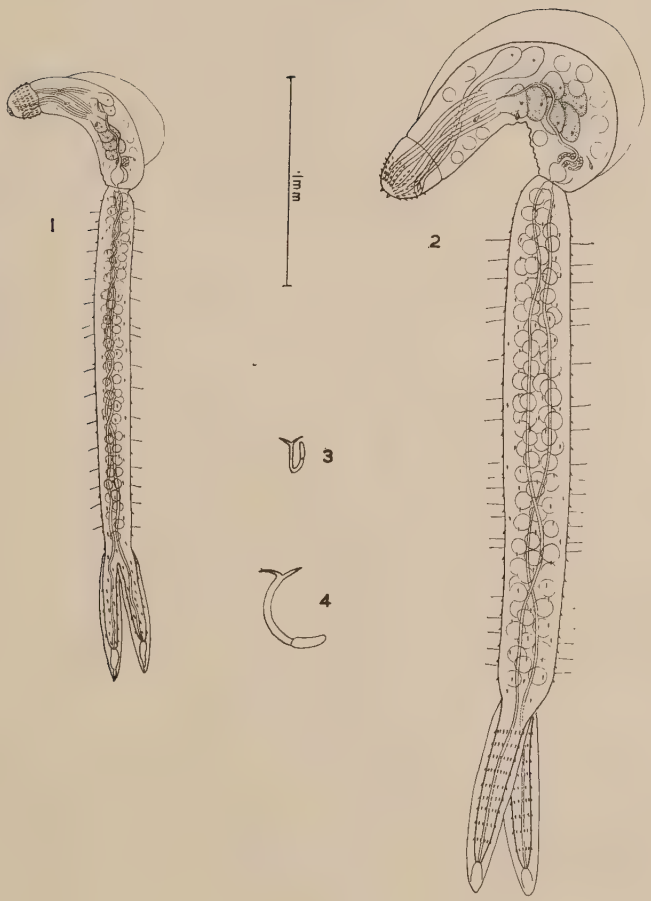


Fig. 1.—General structure of *C. kentensis* n.sp., musculature of the tail shaft omitted. Fig. 2.—General structure of *C. cristocorpa* n.sp., musculature of the tail shaft omitted. Fig. 3.—Resting position of *C. kentensis* n.sp. Fig. 4.—Resting position of *C. cristocorpa* n.sp.

Measurements of Cercaria
(All measurements in millimetres)

	Living		Fixed	
	Range	Average	Range	Average
Body length	0·110–0·130	0·124	0·083–0·100	0·093
Body depth	0·023–0·030	0·025	0·016–0·023	0·020
Tail shaft length	0·216–0·233	0·225	0·166–0·186	0·182
Tail shaft breadth	0·021–0·026	0·023	0·012–0·020	0·018
Furca length	0·086–0·100	0·090	0·060–0·066	0·063
Furca breadth	0·013–0·016	0·014	0·010–0·013	0·011
Anterior organ length	0·016–0·023	0·020	0·016–0·020	0·019
Maximum height of body finfold	0·018–0·02	0·019		

Comparison With Related Species

C. Indicae XIII Sewell, 1922; *C. Indicae XXXIX* Sewell, 1922; *C. Indicae LV* Sewell, 1922; *C. ferrisia* Porter, 1938 and *C. indicanonoides* Porter, 1938 can all readily be separated from the present species in their possession of eyespots.

C. cristata La Val., 1855; *C. microcristata* Ercol., 1881, *C. sanguinicola inermis* Scheuring, 1923; *C.* species from *B. leechi* Ejsmont, 1926 and *C.* species from *L. stagnalis* Ejsmont, 1926, all have their body finfolds extending over the full length of the body behind the anterior organ, and have a rhabdocoele gut. Both these characters separate these species from the cercaria under study, which has reduced body finfold and no gut at all. *C. sanguinicola inermis* also differs in that its furcal finfolds extend over the terminal part of the tail shaft.

In the reduction of body finfold the present cercaria agrees with *C. helvetica XVI* Dubois, 1929; *C. lophosoma* Johnstone and Beckwith, 1947 and *C. muda* Fain, 1953. The last of these can readily be separated from the present species in its number of flame cells, in having an alimentary canal and single caudal excretory duct in the tail shaft. The description of *C. helvetica XVI* is very brief. There is no mention of flagellets on its tail shaft, details of spination or gut in the body. However this species can be separated from the present cercaria by the reduction of the finfold on the furca, and a longer tail stem and much shorter furca in the former, but a detailed comparison is not possible for want of further knowledge about Dubois's species.

C. lophosoma agrees very closely with the present species in size, except for a slightly longer tail shaft in the former. These two species also agree in complete absence of a gut. But *C. lophosoma* differs in lacking anterior conical processes and in having a single caudal excretory duct in the tail shaft. These two species develop in widely different snail hosts. *C. lophosoma* further differs from the present species in that it develops in nearly rounded sporocysts, while the latter develops in elongated sporocysts.

The present species is therefore regarded as new and the name *Cercaria kentensis* is proposed for it.

Cercaria cristocorpa n.sp.

This cercaria was found only once. Out of ten *Valvata piscinalis* (Müll.) collected from Bushy Park on 20th September 1959, one was found to be discharging this cercaria.

Sporocyst

Development takes place in small, oval or sub-circular sporocysts in the digestive gland of the host. The sporocysts are very delicate and measure 0·12–0·195 × 0·075–0·105 mm. The birth pore is absent.

Emergence and Behaviour

Unlike *C. kentensis* this cercaria was never found to be discharged during the day, but whenever the snail was isolated in a tube and left overnight, a large number of cercariae were found to have been discharged during the night. As in *C. kentensis* this cercaria hangs motionless in water for a long time if undisturbed, followed by a very short swimming period, in which it darts for a short distance, tail foremost. The resting posture in this case is different from *C. kentensis*. In this case the furca are kept up but only slightly open and the tail shaft is also only slightly bent, keeping the furca, tail shaft and body in a semi-circle, the body never touching the tail.

The cercariae are usually well distributed in the water, but they show a weak tendency to aggregate near the upper level.

Cercaria

The highly contractile body is kept in the living stage in the form of a semi-circle. There is a dorsal finfold starting just behind

the anterior organ and extending to the junction of the body with the tail. Protrusible snout-like anterior organ is clearly demarcated from the body by a transverse constriction. There is a pair of conical projections, at the anterior tip of the anterior organ. After a bare circum-oral area five rows of prominent spines encircle the anterior half of the anterior organ. Rest of the body is aspinose. There is a large number of cells with granular contents and nuclei, in the body. Their exact number could not be counted, but at least eight glands have been seen. Of these six form a posterior group, with their ducts opening at the tip of anterior organ, while two pairs of glands occupy a position at the middle of body and in front of it on the dorsal side, each of these pairs is connected with a duct, which runs obliquely in the body to open in the middle of anterior organ on the ventral side.

There is no trace of an alimentary canal. The excretory system and the reproductive primordium is similar to that described for *C. kentensis*.

Tail shaft is long, with numerous rounded cells forming its core, these cells are irregularly arranged. Spines on the tail shaft are comparatively more prominent but irregularly arranged. There are eighteen long hair like structures, the flagellets, on each side of the tail shaft. Furca are laterally compressed, with a finfold extending the entire length and continuous over its tip. The spines on the furca are prominent and are arranged in nine characteristic parallel rows. Several oblique muscles, in the tail shaft, originate at the lateral margin and terminate near the centre.

Measurements of Cercaria
(All measurements in millimetres)

	Living		Fixed	
	Range	Average	Range	Average
Body length	0·166–0·200	0·172	0·123–0·146	0·136
Body depth	0·050–0·066	0·060	0.031–0·041	0·036
Tail shaft length	0·283–0·316	0·300	0·240–0·250	0·245
Tail shaft breadth	0·025–0·040	0·033	0·023–0·033	0·026
Furca length	0·123–0·140	0·130	0·086–0·096	0·091
Furca breadth	0·018–0·023	0·022	0·013–0·020	0·016
Anterior organ	0·023–0·036 ×	0·030 ×	0·026– ×	0·026 ×
	0·030–0·036	0·033	0·018–0·021	0·020
Height of body finfold	0·020–0·023	0·022	0·010–0·020	0·015

COMPARISON WITH RELATED SPECIES

This cercaria differs from *C. kentensis* in its larger size, presence of an unreduced body finfold, number of rows of spines on anterior organ, their arrangement on furca, shape and size of sporocyst and the posture during the resting period. They also develop in quite different hosts.

C. cristata as described by Wesenberg-Lund (1934), *C. microcristata*, *C. helvetica XVI*, *C. sanguinicola inermis* and *C. lophosoma* all are smaller than the present species. The first two of these also differ in having a gut, which is completely absent in the present species, and the arrangement of spines on furca. *C. sanguinicola inermis* further differs in having its furcal finfold extending over the terminal part of tail shaft. *C. lophosoma* does not have anterior conical processes, which occur in the present species. It also differs in having a reduced body finfold and arrangement of the furcal spines and in having a single caudal excretory duct. *C. helvetica XVI* is very briefly described, but can further be differentiated from the present species by its reduction of finfold on body and furca. *C. cristata* according to La Valette's (1855) original measurements has a much larger tail shaft, but much shorter furca, while according to Ercolani's (1881) measurements it has much larger body, tail shaft and furca. According to Wesenberg-Lund's (1934) measurements it is smaller than the present species. However, it further differs from the present cercaria in developing in elongated sporocysts.

C. species from *B. leechi* has a smaller body but a longer tail shaft. It also has a rhabdocoele gut which is absent in the present species. *C.* species from *L. stagnalis* has smaller body and furca but a longer tail shaft. It also differs from the present species in having a gut.

C. muda has a larger number of flame cells in the body, a reduced body finfold, single caudal excretory duct, an alimentary canal, and a much larger number of anterior conical processes. All of these characters readily differentiate it from the present species.

It is proposed to name the present species, which is new, as *Cercaria cristocorpa*.

SUMMARY

Representatives of the "Lophocercous" group are recorded from Britain for the first time. Two new species are described and a summary of the classification of this group is discussed.

References

Croft, J. W., 1933.—"A description of *Cercaria whitentoni* n.sp." *Trans. Amer. micr. Soc.* **52,** 259–266. (W.L. 21400).

Dawes, B., 1946.—"The Trematoda, with special reference to British and other European forms." Cambridge University Press. 644 pp.

Dubois, G., 1929.—"Les Cercaires de la région de Neuchâtel." *Bull. Soc. neuchâtel Sci. nat.* **53,** 1–117. (W.L. 5299).

Ejsmont, L., 1926.—"Morphologische, systematische und entwicklungsgeschichtliche Untersuchungen an Arten des Generes *Sanguinicola* Plehn." *Bull. internat. Acad. cracovie* (*Acad. pol. sci.*) (ser. B.) 877–966. (W.L. 4346).

Ercolani, G., 1881.—"Dell' adattamento della specie all' ambiente. Nuove richerche sulla storia genetica dei Trematodi." *Mem. Accad. Sci. Inst. Bologna.* **2,** 1–334.

———, 1882.—"Dell' adattamento della specie all' ambiente. Nuove richerche sulla storia genetica dei Trematodi." *Ibid.* **3,** 43–111.

Fain, A., 1953.—"Contribution a l'étude des formes larvaires des Trematodes au Congo belge et spécialement de la larve de *Schistosoma mansoni*." *Mem. Inst. colon. belge. Sci. nat.* **22,** 1–312. (W.L. 132466).

Johnston, T. H. and Beckwirth, A. C., 1947.—"Larval trematodes from Australian freshwater molluscs. Part XIII." *Trans. roy. Soc. S. Aust.* **71,** 324–333. (W.L. 21654).

Krull, W. H., 1934.—"Some observations on the cercaria and redia of a species of Clinostomum, apparently *C. marginatum* (Rudolphi, 1819) (Trematoda : Clinostomidae)." *Proc. helm. Soc. Wash.* **1,** 34–35. (W.L. 16747a).

Lühe, M., 1909.—"Parasitische Plattwürmer. I. Trematoden." (in Die Süsswasser Fauna Deutschlands). **17,** 1–217.

McCoy, O. R., 1929.—"Notes on cercariae from Missouri." *J. Parasit.* **25,** 199–207. (W.L. 11428).

Miller, Jr., H. M., 1923.—"Comparative studies on Furcocercous cercariae." *Illinois biol. Monogr.* **10,** 1–112. (W.L. 9822).

Odhner, T., 1911.—"*Sanguinicola* M. Plehn—eine digenetischer Trematoden." *Zool. Jb., Syst.* **31,** 33–45. (W.L. 23831).

Porter, A., 1938.—"The larval trematodes found in certain South African mollusca, with special reference to Schistosomiasis (Bilharziasis). *Publ. S. Afr. Inst. med. Res.* No. 42 (Vol. 8), 1–492. (W.L. 17416).

Scheuring, L., 1923.—"Der Lebenszyclus von *Sanguinicola inermis* Plehn." *Zool. Jb.* **44,** 265–310. (W.L. 2383).

Sewell, R. B. S., 1922.—"Cercariae Indicae." *Ind. J. med. Res.* **10,** suppl. 1–370. (W.L. 9940).

Soparker, M. B., 1921.—"Notes on some furcocercous cercariae from Bombay." *Ibid.* **9,** 23–32. (W.L. 9940).

Wall, L. D., 1939.—"Life history of *Spirorchis* sp. (Trematoda : Spirorchiidae)." *J. Parasit.*, **25,** suppl. 28. (W.L. 11428).

———, 1940.—"Life history of *Spirorchis parvus* (Stunkard) Trematoda : Spirorchiidae." *Science,* **92,** 362–363. (W.L. 19938).

———, 1941.—"Life history of *Spirorchis elephantis* (Cort, 1917), a new blood fluke from *Chrysemys picta*." *Amer. Midl. Nat.* **25,** 402–412.

———, 1941.—"*Spirorchis parvus* (Stunkard) its life history and the development of its excretory system (Trematoda : Spirorchiidae)." *Trans. Amer. micr. Soc.* **60,** 221–260. (W.L. 21400).

Wesenberg-Lund, C., 1934.—"Contribution to the development of Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters." *D. Kgl. Danske Vidensk. Selsk Skrifter. naturv.* **9,** 1–223. (W.L. 11681).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 143–160.

On *Dipetalonema manson-bahri* n.sp., from the Spring-hare, *Pedetes surdaster larvalis*, with a Note on its Development in Fleas

By George S. Nelson, M.D., D.T.M., D.A.P. & E.

Division of Insect-borne Diseases,
Medical Research Laboratory, Nairobi

Pedetes surdaster is locally prevalent in the dry grasslands on the Kenya highlands. It is strictly nocturnal and is rarely seen except in the headlights of a car. It is a burrowing animal regarded as a nuisance by farmers. Blood slides taken from *Pedetes* on the Athi Plains near Nairobi were found to contain microfilariae and adult worms of the *Dipetalonema* type were subsequently found in several animals. The only previous record of a filarial parasite from a spring-hare is of *Filaria martis* Gmelin, 1790, found by Anderson (1960) in a *Pedetes caffer* from South Africa.

The Adult Worms

Eight males and ten females were removed from two spring-hares. They were found in the subcutaneous tissue and fascial sheaths overlying the muscles of the hind limbs and back. The animals had very little subcutaneous fat and this greatly facilitated the search for worms. They were seen by the naked eye lying coiled up in the tissue spaces rather like *Dirofilaria repens* in cats or *D. corynodes* in monkeys, but they were much smaller.

The worms were dissected out with a probe and blunt forceps, washed in saline and then killed with hot 70% alcohol and preserved in glycerine alcohol. Permanent preparations for detailed examination were made using the hanging-drop technique as described for *Brugia* spp. by Buckley and Edeson (1956).

There was no evidence of pathogenicity ; the worms were free in the tissue spaces. One worm was completely calcified.

Morphology

They are medium sized worms with a smooth cuticle (see Table I for dimensions). The males are about half the size of the females which averaged 56·9 mm. in length. The head is globular and

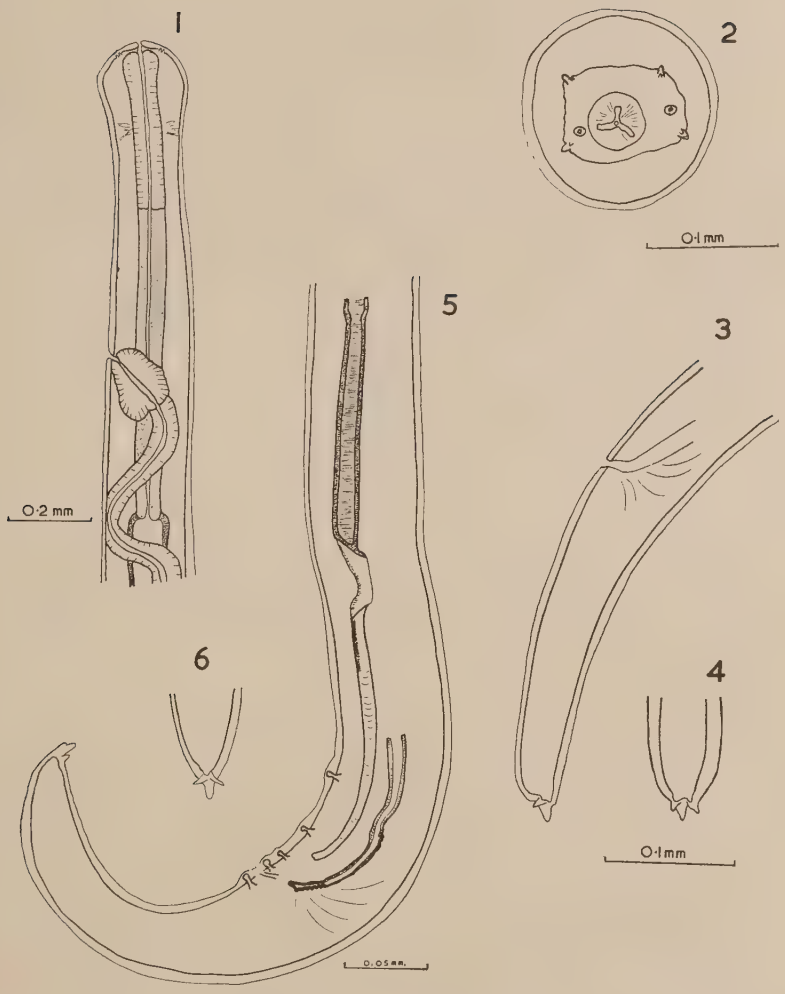
narrows to a neck. The *en face* view shows that the mouth is triangular; that there are two lateral amphids and four pairs of lateral papillae, each pair comprising one large external papilla and one small internal one. (Fig. 2). The tail has a prominent terminal conical projection with two sub-terminal ventro-lateral digits.

TABLE I

Measurements of 12 adult *Dipetalonema manson-bahri* n.sp.

FEMALE :	No. 1	2	3	4	5	6	Mean
Length	42·5	63·5	59·5	61·6	51·0	63·5	56·9 mm.
Max. Breadth	155	240	260	190	250	210	217μ
Head Diam.	140	140	140	90	130	140	137μ
Ant. oesophagus	345	370	450	—	450	370	397μ
Post oesophagus	825	820	—	—	750	900	822μ
Total oesophagus	1170	1190	—	—	1200	1270	1219μ
Nerve Ring	205	200	210	—	210	210	207μ
Vulva	670	750	980	—	800	750	790μ
Tail	245	280	260	—	280	250	263μ
MALE :							
Length	30·0	24·0	27·5	23·5	28·5	28·5	27·0 mm.
Max. Breadth	140	150	150	140	130	150	143μ
Head Diam.	80	90	85	105	80	80	87μ
Ant. oesophagus	450	450	370	380	320	370	388μ
Post oesophagus	750	750	650	800	800	820	761μ
Total oesophagus	1200	1200	1020	1170	1120	1190	1149μ
Left Spicule	338	325	250	320	415	370	336μ
Right Spicule	128	98	95	105	130	135	115μ
Spicule ratio	2·6 : 1	3·3 : 1	2·6 : 1	3·0 : 1	3·2 : 1	2·7 : 1	2·9 : 1
Tail	210	195	180	192	135	170	180μ

Female : The oesophagus is divided into a short anterior muscular part and a longer posterior glandular part. The vulva orifice is in the mid-oesophageal region (Fig. 1) and opens from a prominent



Dipetalonema manson-bahri n.sp.

Fig. 1.—Anterior end of female. Fig. 2.—Anterior end, female, en face. Fig. 3.—Female tail. Fig. 4.—Caudal extremity, female. Fig. 5.—Male tail. Fig. 6.—Caudal extremity, male.

ovijector. The tail is slightly curved and tapers to a rounded extremity with three terminal projections ; the central projection is dorsal, fleshy and very prominent and the other two are ventral, less conspicuous and project laterally like small triangular ears. (Fig. 4).

Male : The anterior extremity of the male is similar to that in the female. There are usually three coils to the corkscrew shaped tail which is more pointed than in the female but it also has a trilobed extremity. (Fig. 6). There are four pairs of precloacal papillae and one pair of postcloacal papillae. The left spicule is in three parts : there is a stout, tubular, open-ended handle, a short twisted central portion and a narrower long terminal blade. In one specimen the left spicule was extruded, and it was seen to be covered by a fine sheath. The right spicule is short. It is poorly defined proximally. The distal third is narrow and corrugated. There is no gubernaculum. (Fig. 5).

The Microfilaria

The microfilariae were examined in fresh preparations, in 2% formalin and in preparations stained with either methylene blue, Giemsa, haematoxylin or pyronin methyl green. They are unsheathed. In fresh blood under a coverslip they are very active with a snake-like movement and they show no tendency to stick to the coverslip.

In 2% formalin they died extended with only a slight curve to the tail ; often the head was flexed about 8μ from the end. The dimensions of the microfilariae in formalin were very constant averaging 262μ by 4·8μ. Using the same technique Newton and Wright (1956) found that the microfilariae of *Dirofilaria immitis* measured 313μ×6·9μ and those of the dog *Dipetalonema* (? *reconditum*) measured 276μ×4·6μ.

The characteristic features of the microfilariae were best seen in thick films which had been left overnight, then dehaemoglobinised in water, fixed with methyl alcohol and stained for 1 hour with 1 in 10 Revector Giemsa with a 6·5 pH buffer. By this method there was some contraction of the microfilariae which now measured 237μ×6·3μ. The cephalic space was relatively long averaging 10·5μ, and in many specimens the "Mundegebilde" was very distinct. (Fig. 7). This structure was illustrated by Fülleborn (1913) as a prominent feature in the microfilariae of *Loa loa* ; it was seen by

Vogel (1928) in *D. perstans* and by Feng (1933) in *Brugia malayi*. It seems likely that this structure is attached to the hooklet which develops in the first stage larvae.

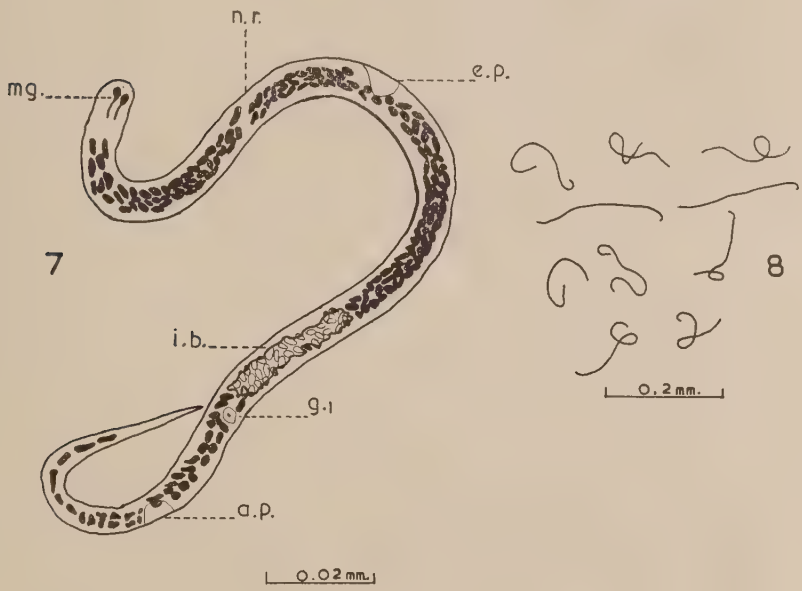


Fig. 7.—Microfilaria of *Dipetalonema manson-bahri* n.sp.

mg.—"Mundegebilde"; **I.b.**—Inner body; **nr.**—Neural ring; **Gl.**—First "G" cell; **e.p.**—Excretory pore; **a.p.**—Anal pore.

Fig. 8.—Microfilariae under low magnification to show "attitudes".

The last 7 or 8 nucleii are in a single row; the last three are usually attenuated. The tail tapers to a blunt point and is free of nuclei for the last 17μ. The neural ring, excretory pore and genital pore are all clearly seen but the G cells are difficult to distinguish. A striking feature was the deep-red staining "inner body" which obscured the nuclei from about 130μ to 165μ from the anterior end.

The microfilariae are shorter but are very similar to those of *Dipetalonema arbuta* Highby, 1943, a parasite of the North American porcupine.

Type host :	*Pedetes surdaster larvalis.*
Other hosts :	Unknown.
Habitat :	Subcutaneous connective tissue and fascial spaces overlying the hind limbs and back.
Locality :	Leukenia Farm, Athi Plains, nr. Nairobi, Kenya.
Types :	Deposited in the Helminthological Collection of the Department of Parasitology, London School of Hygiene and Tropical Medicine.

The species is named for Sir Philip Manson-Bahr as a tribute to his outstanding contribution to the study of filariasis.

Relationships

The genus *Dipetalonema s.l.* was shown by Yeh (1957) to contain "some forty species with a most diverse collection of heterogeneous characters". He tentatively suggested that they could be arranged in 7 genera. The genus *Dipetalonema* was to be reserved for only two species, *D. caudispina* (Molin, 1858) and *D. gracile* (Rudolphi, 1809), both parasites of primates. In Yeh's classification the present species would be accommodated in the genus *Acanthocheilonema* Cobbold, 1870. The distal section of the left spicule is, however, much less slender than the other species assigned to this genus, which would include *A. arbuta* (Highby, 1943), *A. bifida* (Molin, 1858), *A. diacantha* (Molin, 1858), *A. finlayi* (Mazza and Fiora, 1932), *A. sprenti* (Anderson, 1957) and *A. travassosi* (Artigas and Pachco, 1933), species which are also found in Rodentia. Yeh excludes from *Acanthocheilonema* the species *D. vite* (Krepkogorskaya, 1933) which is also found in rodents and includes in it the genus *Monnigofilaria* Skryjabin and Schikhobalowa, 1948. Yeh's classification has not been accepted by Chabaud and Anderson (1959); they still prefer to regard *Acanthocheilonema*, *Monnigofilaria*, *Tetrapetalonema* Faust, 1935, *Loxodontofilaria* Van den Berghe and Gillian, 1939 and *Molinema* Freitas and Lent, 1943 as synonyms of *Dipetalonema* Diesing, 1861. This is still a heterogeneous collection of at least 25 species from Carnivora, Rodentia, Insectivora and Primates.

All attempts at classification of these filaria worms have so far been confined to the grouping of species on the evidence of common characters of adult morphology without reference to host specificity, habitat in the host, larval characters, or the cycle of development in intermediate hosts. Several species are confined to natural orders of mammals ; one group is confined to primates, another to rodents. Some species are confined to the peritoneal cavity, others to the subcutaneous connective tissue. In some the microfilariae are sheathed, in others they are unsheathed ; some have short stumpy microfilariae with nuclei to the tip of the tail, whereas in others the microfilariae are long and thin with an attenuated tail free of nuclei ; In some species the microfilariae are in the skin, in others they are in the blood. The life cycles of many of the species have not been worked out but included in this broad genus are species transmitted by mosquitoes, *Culicoides*, ticks and fleas.

When we have more information on the biology of these parasites we might expect a more generally acceptable classification. For the moment it is perhaps unwise to subdivide the genus. I have, therefore, followed Chabaud and Anderson's classification and included the present species in the genus *Dipetalonema* Diesing, 1861.

The Development of *D. manson-bahri* n.sp. in Fleas

Infective larvae of the *Dipetalonema* type were found in 2 out of 29 *Delopsylla crassipes* collected from a spring-hare near Nairobi. (*D. crassipes* is only found on *Pedetes surdaster*). It was not possible to make further observations on this insect in Kenya but the author has had an opportunity of following up this observation by feeding laboratory bred fleas on an infected spring-hare in London. Complete development to infective larvae was seen in both *Ctenocephalides felis* and *Xenopsylla cheopis*.

Material and Methods

All feeding experiments were carried out on an adult male spring-hare which was caught on Leukenia Farm, near Nairobi and flown to England. Microfilarial densities in this animal varied markedly from day to day ; the lowest level was 73 Mf. per 20 cu. mm., the highest 470 per 20 cu. mm. No full periodicity studies have been made but it was noted that microfilarial densities were highest at night and under chloroform anaesthesia.

The animal was wild and for several weeks it was very difficult to handle. It is as big as a rabbit and has very short fore legs and very long hind legs ; hence it is sometimes known as the kangaroo hare. It is in fact quite unrelated to the true hares. It belongs to the Order Rodentia, Sub-Order Hystricomorpha, Family Pedetidae. Its nearest relatives are the Hystricidae (porcupine) ; the Thyronomyidae (cane rats) and the Petromyidae (dassie rats) all belonging to an ancient relict group.

The male animal was accompanied by an uninfected female. The authorities at the London Zoo were pessimistic about the chances of survival. They have been fed on oats "Diet 86" pellets and occasional fresh vegetables and kept in ordinary rabbit cages. A month after arrival in London the female produced an offspring which is now nearly fully grown.

It was hoped to study the development of the filaria in *Delopsylla crassipes* but although the animals were infected with this flea when they left Nairobi they were free of fleas on arrival in London ; it is possible that this was due to insecticide spraying of the aircraft. Six attempts were made to send living *D. crassipes* to London by air but they all died en route. On one occasion a batch of *C. felis* survived the journey.

It was decided to try to infect local fleas. A culture of *X. cheopis* was available but all preliminary experiments with this species were unsuccessful. If the fleas were left on the animal for less than 24 hours they failed to pick up microfilariae and if left for more than 24 hours they were usually all dead. It was decided that a more robust flea like *C. felis* might give a better result but no culture of *C. felis* was available in England and very few fleas could be found on dogs and cats even in "The Home" for stray animals at Battersea. This was in winter ; I was assured that fleas would not be prevalent until mid-summer. Fortunately, thanks to Dr. M. M. J. Lavoipierre of the Kaiser Institute Laboratory of Medical Entomology in California, a supply of *C. felis* pupae was sent from America.

The author had very little experience in handling fleas ; it was found that techniques used in previous work on mosquito transmission of filaria were of little value. Before a satisfactory technique was developed more than 400 *X. cheopis* and more than 1,000 *C. felis* were fed on the infected animal ; 300 fleas were dissected in the course of eleven preliminary experiments.

At first it was thought that all that was necessary was to feed the fleas on the animal for a few hours and then keep them at a constant temperature and humidity and dissect specimens daily.

But even when there were more than 20 Mf. per cu. mm. in the animal's blood, few fleas picked up even one microfilaria during the period practicable for holding or anaesthetising the animal. The animal would not allow a container with a fine mesh to be strapped to its body. Eventually we thought we had the solution to the problem when a wide-bore polythene tube containing fleas was fitted onto the tail of the animal; but too often it was either torn off or if too tightly applied it produced an ooze of serum through the flea bites and the enclosed fleas were drowned. When the fleas were allowed to wander freely on the animal in its cage they had nearly all disappeared within 24 hours. Another problem was that fleas that had picked up the infection showed very little sign of development of the larvae when they were kept either individually or in small groups in tubes, conical flasks or sweet jars. The fleas survived and the microfilariae penetrated the fat body cells but they failed to develop beyond the first stage. The fat body got smaller and all development ceased.

It was obvious that if we were to obtain adequate numbers of larvae developing in the fleas, two conditions were necessary: (1) The fleas must feed on the hare for more than 24 hours, (2) they must be provided with a constant supply of food to maintain the fat body.

Eventually a fairly simple technique was found. The hare was put into a smooth sided plastic dustbin with a one inch layer of sand, oats and vermiculite in the bottom. Adequate ventilation was provided by boring holes in the lid and tying two spars under the lid. Without adequate ventilation there was condensation of moisture on the sides of the bin and the majority of fleas were dead the following morning. To avoid this during the experiment the hare was fed on dried oats which decreased the quantity of urine produced; the relative humidity in the room was reduced by means of an electric heater.

The fleas were put direct on the animal and left to feed for a minimum of 72 hours. With an average of 10 Mf. per cu. mm. of the animal's blood the intake of fleas was approximately 1 Mf. in 24 hours, 3 Mf. in 48 hours, 6 Mf. in 72 hours and 10 Mf. in 96 hours. It would have been an advantage to have left some of the fleas on the hare for the full period of development but when this was done only 20 per cent of the original fleas were alive after 96 hours and at the end of one week all the fleas were dead.

In Kenya we found that *C. felis* became infected with *Dipetalonema* of the dog even when the microfilarial densities were

less than 1 in 50 cu. mm. In fact the fleas provided an excellent example of xenodiagnosis provided they remained on the host for more than one week.

Infected fleas were collected off the spring-hare and from the sand-oats-vermiculite mixture by using a water suction pump or by simple mouth suction into test tubes. It was found helpful to tip the debris from the bottom of the dustbin into a glass tank having two sides blackened, whence the fleas were then easily collected from the dark corner. *X. cheopis* was easier to handle than *C. felis*, as it does not jump so high.

The infected fleas were then transferred to "sweet jars" (11 in. $\times$ 6 in. $\times$ 4 in.) which contained a 1 in. layer of the sand-oats-vermiculite mixture. A white mouse, in a wire restraining cage, was provided as a constant source of food for the fleas. Both species fed more easily on the mice than on the hare. The sweet jars were kept in a constant temperature room at 28°C. with a relative humidity varying between 50 and 75%. The majority of the fleas survived for at least three weeks under these conditions.

In *X. cheopis* there was a smooth progressive development of larvae which reached the infective stage between the 15th and 18th days. One flea contained 24 fully mature infective larvae and 100% of fleas that had fed on the hare for more than 72 hours developed infective larvae.

Unfortunately the supply of *C. felis* was exhausted before this technique was perfected but some that had escaped* onto a cat in a neighbouring cage after feeding on the hare were dissected 17 days later and found to contain infective larvae identical with those seen in *X. cheopis*. The initial stages of development in *C. felis* were also identical with those in *X. cheopis*. It is probable that it is also an efficient intermediate host.

Female fleas of both species ingested more microfilariae and developed far larger fat bodies than male fleas. The larvae seemed in no way to interfere with the development of eggs. It is interesting to note that during the first 10 days of emergence from pupae all the *C. felis* were females. Insufficient observations were made on the differences in development in male and female fleas but it was noted that although the larvae reached the infective stage in both sexes development was delayed in the males.

* The cat was infected with *Brugia patei*; the microfilariae of this species were plentiful in the haemocoale of the fleas but none had migrated to the thorax and none showed any sign of development.

The Development of *D. manson-bahri* n.sp. in *Xenopsylla cheopis*

It was not possible to study the route of migration of the microfilariae from the gut to the haemocele because the fleas ingested too few microfilariae and only on one occasion were they seen in the mid-gut. Usually, when first dissected, the microfilariae were either free in the haemocele or already established in abdominal fat body cells. No microfilariae or any developing stages were ever found in the malpighian tubules, the rectum, the ovarioles, the muscles of thorax and legs, or in the head.

All development appeared to take place in the large fat body cells of the abdomen. At times developing larvae were found free in the abdominal haemocele but this was probably due to rupture of the cells during dissection. During the first three days the larvae were very active and were seen to twist and writhe about inside the fat body cells. In fleas newly emerged from pupae the fat body cells were small and compact, full of globules which stained well with Sudan blue. (Fig. 9A). With continuous feeding the fat body cells increased in size and there was progressive development of the larvae but in starved fleas the cells became smaller, lost their globules and the larvae remained in the microfilarial stage for as long as 14 days.

The most striking feature of the microfilariae and first stage larvae in the haemocele and fat body is the small hooklet or curved lancet at the anterior end. (Fig. 9, B, C, D and Fig. 13). This was never seen in the blood forms. A similar structure was seen in the first stage larvae of *Dipetalonema vite* by Chabaud (1954) in ticks; he says, "L'extrémité céphalique porte une épine en forme de crochet rabattu en arrière, qui est très bien visible sur le vivant". It is possible that this structure is used for penetrating the gut and for entering the fat body cells; it is shed with the first ecdysis. About the third day the majority of larvae are shorter and fatter ($200\mu \times 10\mu$); the excretory cell and genital cells are very prominent; faint cuticular striations are often visible. No marked "sausage" stage was observed. The "sausage" stage is a characteristic feature of the development of most filarial worms but Chabaud (*loc. cit.*) found no "sausage" stage in *D. vite*; Pandit, Pandit and Iyer (1926) make no mention of it in the development of *Conspiculum guindiensis* and Noé (1908) did not find it in *D. grassii*.

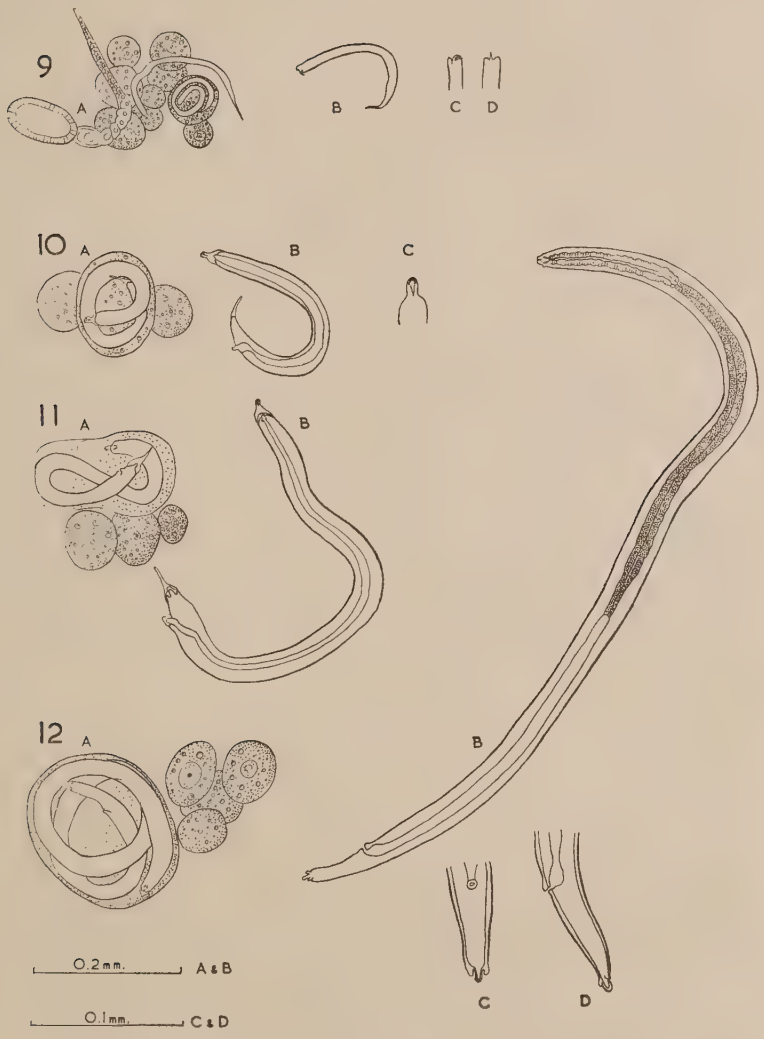
The first stage larva develops a distinct rounded anterior projection which is also present in the second stage (Fig. 10 B. and C.).

A similar structure was illustrated by Grassi and Calandruccio (1890) in the larvae of *Dipetalonema reconditum* and by Chabaud (*loc. cit.*) in *D. vite*.

By the seventh day the larvae are longer and much fatter, averaging 350μ × 25μ ; there is a prominent anal plug and a primitive alimentary canal. The cuticle of the first stage larva is free at both ends. Anteriorly the first larval cuticle with its proboscis and hooklet are visible ; and posteriorly the nucleus-free extremity of the microfilaria is seen as a short, clear "tail". (Fig. 10 B). In the corresponding stage of development in *Brugia patei* this "tail" contains the two nuclei of the microfilaria.

The second stage larvae increase rapidly in size and on the 10th day measure 700μ × 30μ. The host fat body cell is greatly distended and most of the fat globules have disappeared. The first larval cuticle is still attached and in some specimens there is evidence of a commencing second ecdysis. Anteriorly a buccal capsule may be visible and posteriorly the terminal lobes are beginning to form.

Infective third stage larvae were first seen on the 15th day. The majority are free in the abdominal haemocele and can be seen moving actively even before the flea is dissected if examined by transmitted light. Some fully developed third stage larvae were still inside the stretched envelopes of fat body cells (Fig. 12 A). The larvae were confined to the abdomen. Specimens fixed in glycerine alcohol and mounted in glycerine, after slow evaporation of the glycerine, varied from 1150μ to 1435μ in length. They tapered at both ends, the maximum breadth was at the junction of the anterior third and posterior two-thirds and varied from 27μ to 29μ. A ring of papillae is present a short distance from the anterior extremity. There is a well marked short buccal cavity. The oesophagus is divided into an anterior muscular part 150μ long and a posterior glandular part 550μ long. The internal structures are best seen in living specimens. (Fig. 12 B). The anus is on a slight elevation, and the tail narrows markedly to the caudal extremity which is formed by a terminal conical projection and two ventro-lateral digits, rather like the caudal extremity of the adult worm. The tail averaged 65μ in length and 17μ in breadth giving an anal ratio of 4. These larvae are indistinguishable from those found in naturally infected *D. crassipes*. They are very similar to the infective larvae of *Dipetalonema reconditum* illustrated by Calandruccio (1892) and of *D. arbuta* illustrated by Highby (1943). Chabaud (*loc. cit.*) was able to distinguish male and female infective larvae of *D. vite* ; although the larvae of *D. manson-bahri* are very similar it has not been possible to differentiate the sexes.



The Development of *D. manson-bahri* n.sp., in *Xenopsylla cheopis*.

Fig. 9.—*First stage larvae.* A. Free in haemocele and in fat body cell of young flea. B. Larva with hooklet. C. Lateral view of head. D. Dorso ventral view of head. Fig. 10.—*Second stage larvae.* A. In fat body cell. B. Free. C. Hooklet of 1st stage on anterior extremity of shed cuticle. Fig. 11.—*Late Second Stage Larvae.* A. In fat body. B. Free. Fig. 12.—*Third Stage Larvae.* A. In fat body cell. B. Free. C. Ventral view of caudal end of infective larva. D. Lateral view.

Discussion

Lavoipierre (1958) in a very valuable review of the literature dealing with the development of filarial worms in arthropods, lists 33 species with known intermediate hosts, the majority developing in mosquitoes and only one developing in fleas. To this list can be added four species transmitted by mosquitoes : *Brugia patei* and *Setaria equina* (Heisch, Nelson and Furlong, 1959) ; *Dirofilaria magnilarvatum* (Wharton, 1959) ; and an undescribed species *Microfilaria fijiensis* (Symes and Mataika, 1959). The present species, *D. manson-bahri* developing in fleas brings the number of known life cycles to 38.

Following the discovery by Manson (1878) of the development of *W. bancrofti* in mosquitoes, one of the earliest life cycles to be studied was that of *D. reconditum* which was shown by Grassi and Calandruccio (1890) to develop in fleas. This important work was almost forgotten until Newton and Wright (*loc. cit.*) showed that the evidence of Breinl (1921), Brown and Sheldon (1940), Summers (1940) and Steuben (1954) incriminating fleas as vectors of *Dirofilaria immitis* was unacceptable. Newton and Wright (*loc. cit.*) found that dogs in America were often infected with both *Dirofilaria immitis* and *Dipetalonema* (? *reconditum*) ; only the latter species developed in fleas. Much of the literature on the transmission of *D. immitis* requires re-examination in the light of these observations.

Heisch, Nelson and Furlong (*loc. cit.*) found that *D. immitis* on the Kenya coast developed well in local *Aedes aegypti* but "*D. immitis*" from Nairobi would not develop in Nairobi *Aedes aegypti*. It was thought that differences in the strain of vector might explain this result as shown by Roubaud (1937) and Kartman (1953). It is now known that the dogs in the Nairobi area are infected with a *Dipetalonema* and not *D. immitis* ; the microfilariae are superficially very similar. It has also been shown that the dog *Dipetalonema* develops in *C. felis* and that the infective larvae are easily distinguished from those of *D. immitis*. It was the discovery of these typical *Dipetalonema* infective larvae in the fleas which first indicated that this was not *D. immitis*.

In determining the intermediate hosts of particular filarial worms a great deal of confusion can arise when animals are simultaneously infected with several species. In Kenya, dogs are infected with *D. immitis, D. repens, Brugia patei, Dipetalonema dracunculoides*, probably *D. reconditum* and an undescribed *Dipetalonema* with

microfilariae confined to the skin. A knowledge of the morphology of the infective larvae has been of assistance in discovering the vectors of some of these species in nature and in helping to interpret the results of laboratory experiments. An account of the morphology of infective larvae from mosquitoes has been given elsewhere (Nelson, 1959).

So far only one species of filaria has been found in *Pedetes surdaster*. The present work suggests that the vector in nature is *Delopsylla crassipes*. Mr. W. Grainger of the Division of Insect-Borne Diseases in Nairobi has collected many *Pedetes* from this area during the past 25 years ; he informs me that they are usually heavily infected with fleas and only one species, *D. crassipes*, has been found on them. A few ticks and lice have been collected off the hares but so far they have not been found infected.

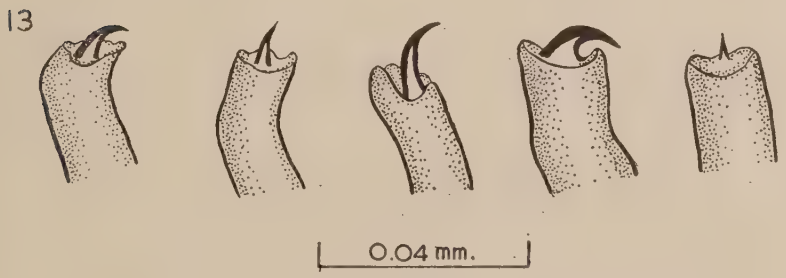


Fig. 13.—Anterior extremity of first stage larvae showing hooklet.

No conclusive record exists of a filarial worm being transmitted by different Orders of arthropods ; usually there is fairly strict intermediate host specificity, limited to the same Family. In Italy, Calandruccio (1892) reported the development of *D. reconditum* in fleas and also in *Rhipicephalus* ticks, but in the same country Noé (1908) found another dog filaria, *D. grassii*, developed in *Rhipicephalus* and it is probable that Calandruccio's dogs had mixed infections. The report by Tao, Wu and Sun (1938) and of Langeron (1938) of *W. bancrofti* in sandflies is a possible exception but the development was incomplete. The observations of Baltazard, Chabaud and Minou (1952) of the development of *D. vite* in both *Ornithodorus* and *Rhipicephalus* is also quite exceptional.

Filarial larvae usually show not only host specificity but tissue specificity with a marked preference for particular cells. For example, *W. bancrofti, Brugia* spp., *Onchocerca* spp., and *Setaria* spp., all develop in thoracic muscles. *Dirofilaria repens, D. immitis* and *D. tenuis* only develop in malpighian tubules; and *Loa loa* and *Dipetalonema reconditum* only in fat body cells. Usually the different species in each genus develop in the same tissues of the same family of arthropods, but there are exceptions especially in the genus *Dipetalonema*: *D. perstans* and *D. streptocerca* develop in the thoracic muscles of *Culicoides*; *D. arbuta* in the thoracic muscles of mosquitoes: *D. vite* in the "skeletal" muscles of hard and soft ticks; *D. grassii* in the muscles of hard ticks; and both *D. reconditum* and *D. manson-bahri* in the fat body of fleas. When there is more information on the life cycle of other members of this genus it may be possible to arrange the species in more generally acceptable groups.

Summary

1. The morphology of the adult worms and the microfilariae are described of a new species of filarial worm, *Dipetalonema manson-bahri.*

2. *Delopsylla crassipes* Jordon, 1926, a flea specific to *Pedetes surdaster,* was found in nature to contain infective larvae which are believed to be those of *D. manson-bahri.*

3. In the laboratory full development of *D. manson-bahri* was observed in *Ctenocephalides felis* and *Xenopsylla cheopis.*

4. The different stages of development in *X. cheopis* are described and illustrated. Development was intracellular and confined to the fat body of the abdomen. No development took place in starved fleas; it was necessary to maintain infected fleas on mice.

5. The first stage has a characteristic anterior hooked lancet. There are two ecdyses. The cuticle of the first and second stage larva is retained until the third stage larva escapes from the fat body cell into the abdominal haemocele.

6. The development of *D. manson-bahri* is very similar to that of the dog filaria *D. reconditum.* A knowledge of the morphology of infective larvae is essential for the identification of natural infections in arthropods and for the correct interpretation of experimental results when animals are infected with several species of filarial worms.

Acknowledgments

I am indebted to Mr. R. Rickman of the Division of Insect-Borne Diseases, Nairobi, for collecting the infected *Pedetes* and for sending the *Delopsylla* to London. I am particularly grateful to Mr. F. R. N. Pester, Mr. P. Blackburn and Mr. S. A. Smith of the London School of Hygiene and Tropical Medicine for their advice and help with the experiments. Dr. M. M. J. Lavoipierre and Dr. E. Benjamini of the Kaiser Foundation Research Institute Laboratory of Medical Entomology, California, generously arranged for a supply of *C. felis*. Dr. L.-S. Yeh has given me the benefit of his incomparable knowledge of the taxonomy of filarial worms.

References

Anderson, R. C., 1960.—"A study of *Filaria martis* Gmelin, 1790 from *Martes foina* and *Pedetes caffer*." *Canad. J. Res.* (*Zool.*), **38,** 157–167. (W.L. 5898c).

Breinl, A., 1921.—"Preliminary note on the development of the larvae of *Dirofilaria immitis* in the dog fleas *Ctenocephalides felis* and *canis*." *Ann. trop. Med. Parasit.*, **14,** 389. (W.L. 1063).

Brown, H. W. and Sheldon, A. J., 1940.—"Natural infections of fleas with the dog heart worm (*Dirofilaria immitis*)." *N. Amer. Vet.*, **21,** 230–231. (W.L. 15224).

Buckley, J. J. C. and Edeson, J. F. B., 1956.—"On the adult morphology of *Wuchereria* sp., (*malayi* ?) from a monkey (*Macaca irus*) and from cats in Malaya and on *Wuchereria pahangi* n.sp., from a dog and cat." *J. Helminth.*, **30,** 1–20. (W.L. 11224c).

Chabaud, A. G., 1954.—"Sur le Cycle Évolutif des Spirurides et de Nématodes ayant une Biologie Comparable." *Ann. Parasit. hum. comp.*, **29,** 206–249. (W.L. 899a).

Chabaud, A. G. and Anderson, R. C., 1959.—"Nouvel essai de Classification des Filaires (Superfamille des Filaroidea) 11.1959." *Ibid.*, **34,** 64–87.

Calandruccio, S., 1892.—"Descrizione degli embrioni e della larve della *Filaria recondita* (Grassi)." *Atti de Accad. gioenia,* **5,** 1–15. (W.L. 2113).

Feng, L. C., 1933.—"A comparative study of the anatomy of *Microfilaria malayi* Brug, 1927 and *Microfilaria bancrofti* Cobbold, 1877." *Chin. med. J.*, **47,** 1214–1246. (W.L. 6177g).

Fullebörn, F., 1913.—"Beitrage zur Morphologie und Differentialdiognose der Microfilarien." *Arch. Schiffs-u Tropenhyg.*, **1,** 7–72. (W.L. 1804).

Grassi, B. and Calandruccio, S., 1890.—"Ueber Haematozoon Lewis; Entwicklungsyklus einer Filaria (*Filaria recondita* Grassi) des Hundes." *Zbl. Bakt.*, **7,** 18–26. (W.L. 23684).

Heisch, R. B., Nelson, G. S. and Furlong, M., 1959.—"Studies on Filariasis in East Africa. I. Filariasis on the Island of Pate." *Trans. R. Roc. trop. Med. Hyg.*, **53,** 41–53. (W.L. 21671).

Highby, P. R., 1943.—"Mosquito vectors and larval development of *Dipetalonema arbuta* Highby (Nematoda) from the porcupine *Erethizon dorsatum*." *J. Parasit.*, **29,** 243–252. (W.L. 11428).

Kartman, L., 1953.—"Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). *Exp. Parasit.*, **2,** 27–28.

LANGERON, M., 1938.—"Notulae zoologicae : Evolution de micro-filaires nocturnes chez les phlébotomes." *Ann. Parasit. hum. comp.*, **16,** 477–478. (W.L. 899a).

LAVOIPIERRE, M. M. J., 1958.—"Studies on the Host-Parasite Relationship of Filarial Nematodes and their Arthropod Hosts. II. The arthropod as a host to the Nematode : A brief appraisal of our present knowledge based on a study of the more important literature from 1878 to 1957." *Ann. trop. Med. Parasit.*, **52,** 326–345. (W.L. 1063).

MANSON, P., 1878.—"On the development of *Filaria sanguinis hominis* and on the mosquito considered as a nurse." *Trans. Linn. Soc., Lond., (Zool.)*, **14,** 304–311. (W.L. 21544).

NELSON, G. S., 1959.—"The Identification of Infective Filarial Larvae in Mosquitoes, with a note on species found in "wild" mosquitoes on the Kenya coast." *J. Helminth.*, **33,** 233–256. (W.L. 11224c).

NEWTON, W. L. and WRIGHT, W. H., 1956.—"The occurrence of a dog Filariid other than *Dirofilaria immitis* in the United States." *J. Parasit.*, **42,** 246–258. (W.L. 11428).

NOÉ, G., 1908.—"Il cyclo evolutivo della *Filaria grassii*, mihi 1907." *R. C. Accad. Lincei*, **17,** 282–291. (W.L. 2118).

PANDIT, C. G., PANDIT, S. R. and IYER, P. V. S., 1926.—"The development of the Filaria *Conispiculum guindiensis* in *C. fatigans* with a note on the transmission of the infection." *Indian J. med. Res.*, **17,** 421–429. (W.L. 9940).

ROUBAUD, E., 1937.—"Nouvelles recherches sur l'infection de moustique fièvre jaune par *Dirofilaria immitis* Leidy. Les races biologique de *Aedes aegypti* et l'infection filarienne." *Bull. Soc. Pat. exot.*, **30,** 511–519. (W.L. 5310).

SUMMERS, W. A., 1943.—"Experimental studies on the larval development of *Dirofilaria immitis* in certain insects." *Amer. J. Hyg.*, **37,** 173–178. (W.L. 600a).

STEUBEN, E. B., 1954.—"Larval development of *Dirofilaria immitis* (Leidy) in fleas." *J. Parasit.*, **40,** 580–589. (W.L. 11428).

SYMES, C. B. and MATAIKA, J. U., 1959.—"Observations on *Microfilaria fljiensis* Yeh, Symes and Mataika, 1958 from fruit bats (*Pteropus hawaiiensis*) in Fiji." *J. Helminth.*, **33,** 223–232. (W.L. 11224c).

VOGEL, H., 1928.—"Zur Anatomie der *Microfilaria perstans*" *Arch. f. Schiffs. u Tropenhyg.*, **32,** 291–306. (W.L. 1804).

WHARTON, R. H., 1959.—"*Dirofilaria magnilarvatum* Price, 1959 (Nematoda : Filarioidea) from *Macaca irus* Cuvier. IV. Notes on larval development in *Mansonioides* mosquitoes." *J. Parasit.*, **45,** 513–518. (W.L. 11428).

YAO, Y. T., WU, C. C. and SUN, C. J., 1938.—"The development of the microfilaria of *Wuchereria bancrofti* in sandfly, *Phlebotomus sergenti* var *mongolensis* : a preliminary report." *Chin. med. J., Suppl.*, **2,** 401. (W.L. 6177g).

YEH, L.-S., 1957.—"On the filarial parasite *Deraiophoronema freitaslenti* n.sp., from the giant anteater, *Myrmecophaga tridactyla* from British Guiana, and a proposed reclassification of *Dipetalonema* and related genera." *Parasitology*, **47,** 196–205. (W.L. 16035).

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, pp. 161–168.

Observations on the Biology and Pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901)

By A. K. Varma, G.B.V.C., Ph.D.

Livestock Research Station, Bihar, Patna, India

In an earlier communication the author (1957) reported on the incidence of *Cotylophoron cotylophorum* as the common amphistome parasite of sheep and goats in India. Several workers (Krull, 1932 and 1934 ; Bennett, 1936 ; Srivastava, 1938 ; Chatterjee, 1931 and 1938 and Sinha, 1950) have studied from time to time the life history of this parasite. Mudaliar (1945) discussed the identity of the cercariae of this trematode and expressed the opinion that *Cercaria indicae* XXVI Sewell, 1922 was perhaps the cercaria of *C. cotylophorum*. Kuppuswamy (1946) dealt with these cercariae and reported that their adults were *Paramphistomum cervi*. This was, however, later amended to *C. cotylophorum* at the instance of the Indian Council of Agricultural Research.

The present studies furnish evidence in confirmation of Mudaliar's view. During the course of this work, which was designed to study the specific pathogenicity of this parasite, some observations were also made on its biology at various developmental stages, particularly in respect of (1) hatching of miracidia, (2) infection of the intermediary mollusc, (3) emergence of cercariae from the infected molluscs and (4) their encystment. Pure infections were established with this parasite in clean lambs, and the clinical and pathological effects were studied ; they are recorded here.

I. Observations on Development

Egg to Miracidium

Living amphistomes were collected at abattoirs, where they were identified by the author, and those belonging to *C. cotylophorum* species were separated out. Some were kept for 3 to 4 hours in watch glasses containing a little filtered tap water, while some were dissected and the eggs removed from their uteri. In the former method the parasites themselves discharged eggs and although they were few in number they were all fertilised ; in the latter method although the supply of eggs was large many appeared to be unfertilised. Eggs

from both lots were kept separately in Petri dishes at room temperature, which varied from 21°C. to 24°C. during the day and 18°C. to 21°C at night. Some were also incubated at controlled temperatures ranging from 24°C. to 30°C. Both lots were examined regularly every day to see the development of the embryos. It was noticed that the Petri dishes containing intra-uterine eggs, showed a very rich protozoal fauna in comparison with the other lot containing only fertilized eggs.

On the 15th day when the Petri dishes containing self-discharged eggs, which had been incubated at 28°C.–30°C., were taken out and examined, a single miracidium was seen swimming about in one Petri dish. This was kept in the room (at 25°C.) for about half an hour and on re-examination a number of actively swimming miracidia was seen. Some filtered tap water was added to the Petri dish and after a little while the supernatant fluid was transferred to another Petri dish along with most of the miracidia. Laboratory bred clean snails were exposed to these miracidia and also to miracidia which were observed the next day in other Petri dishes in this lot.

Hatching commenced on the 18th day in the Petri dishes containing self discharged eggs kept at 26°C. in the incubator (where the temperature fluctuated only slightly). At first only half to one dozen miracidia were seen in each Petri dish. The dishes were left at room temperature (24°C.) for about an hour, and four of them were completely covered with dark paper to exclude light. This was done to see if hatching continued in darkness. After an hour numerous miracidia were seen actively swimming about in all the covered, as well as uncovered, Petri dishes, thus showing that darkness had no adverse effect on the hatching. Petri dishes containing self discharged eggs and kept at room temperature from the beginning of the experiment showed miracidia on the 23rd day.

Intra-uterine eggs kept at 28°C. and 26°C. started hatching from the 23rd day and 27th day respectively ; and those kept at room temperature hatched from the 33rd day.

In some of the Petri dishes it was noticed that some miracidia were mature within the egg-shell ; these Petri dishes were placed in the refrigerator for half a minute. On further examination it was observed that hatching was hastened by this low temperature exposure. This interesting observation was confirmed by repeating the experiments several times ; it was also confirmed when using *Fasciola* eggs.

The eggs and miracidia of *C. cotylophorum* have been fairly fully described by earlier workers.

Miracidium to Snail

Laboratory bred clean snails of different ages and different species viz. *Indoplanorbis exustus*, *Limnaea luteola*, *L. auricularia* var. *rufescens* and *Gyraulus euphraticus* were exposed to miracidia in Petri dishes. In another series of experiments only one snail from each of these four types, all about the same age, was exposed to the miracidia. All these Petri dishes were watched for some time under a wide field binocular microscope. It was noticed that *Indoplanorbis exustus* showed marked attraction for the miracidia, most of which were seen swimming around this particular snail. After about ten minutes some of the miracidia turned around *Gyraulus euphraticus*, but none was ever seen to be attracted to the *Limnaea* spp.

All the snails were kept exposed to the miracidia for about 4 hours and then they were transferred to tanks and maintained in the laboratory by methods described earlier by the author (1954) at the room temperature noted above.

The snails were examined every alternate day after the 15th day of their exposure to the infection, for emergence of cercariae. On the 26th day the *Indoplanorbis exustus* were seen secreting numerous pigmented cercariae. These were studied morphologically and found to be identical with *Cercaria indicae* XXVI Sewell, 1922. Snails belonging to other genera exposed to the infection never secreted any cercariae. A few snails from each type were also dissected daily from the date of exposure to infection for the developing parthenitae. On the second day, i.e. after 24 hours, the miracidium was found in one case (*Indoplanorbis exustus*) to develop into a sac-like sporocyst. After the 4th day small rediae were seen developed inside the sporocyst. On the 6th day free rediae were seen with small cercariae developing inside.

Emergence of Cercariae

The following series of experiments were conducted to study the effect of light, temperature and *p*H on the emergence of cercariae from the snail hosts.

Effect of light and temperature : Infected snails were repeatedly exposed to the effect of light and darkness in separate tubes at known temperatures ranging between 22°C. and 36°C. both in the sun (light) and in incubators (darkness) in order to study the influence of light on the emergence of cercariae ; similar observations were made at night. At the temperatures used in these experiments,

it was noticed that the cercariae were markedly heliotropic. There was absolutely no emergence in darkness, so much so that the profuse emergence noticed in the presence of light ceased entirely under dark conditions. The maximum emergence was noticed in the presence of daylight between the hours 11 a.m. and 2 p.m., provided the temperature and *p*H were suitable. Emergence was also effected under artificial light at the required temperature and *p*H.

In respect of *Fasciola hepatica* cercariae the author (unpublished) had previously observed that they were negatively heliotropic and came out only in the night. Thapar and Tandon (1952 and 1953) also observed that *Fasciola indica* cercariae came out during the night and early hours of the day. Unlike the amphistome cercariae these are known to be non-pigmented and it is probable that this may have something to do with the different heliotropic response of these cercariae.

It was also observed that below 24°C. and above 35°C. there was no cercarial emergence, which commenced at 24°C. and stopped at 36°C. Profuse emergence was noticed between the temperatures 30°C. and 35°C.

*Effect of p*H. Cercariae discharging snails were kept in separate tubes containing watery media of *p*H varying from 2 to 12 and exposed to similar conditions of light and temperature. No cercarial emergence was noticed at *p*H below 3. Between 3·2 to 6·4 a few cercariae came out, but all settled down at the bottom of the tubes and showed very little motility. Between 5·1 and 6·4 the motility gradually increased. Between 6·5 and 9·5 there was profuse cercarial emergence with active motility. Between 9·6 and 11 only a few cercariae came out and showed sluggish motility. The higher the *p*H in this range the slower was the motility. There was no emergence above *p*H 11.

These experiments indicate that these cercariae have a preference for a slightly alkaline medium.

Encystment of Cercariae (*Metacercariae*)

Leaves of the following plants were introduced into the tubes containing cercariae in order to study encystment (Hindi equivalents in parentheses) : *Cyperus rotundus* ("Motha") ; *Ipomoea reptans* ("Karmi") ; *Cynodon dactylon* ("Doob") ; *Hygrorrhiza aristata* ("Danti") ; *Commelina bengalensis* ("Kena") ; *Hordeum vulgare* ("Jau") ; *Hygrophila spinosa* ("Gorkhul") ; *Caesulia axillaris* ("Belonda") ; *Saccharum munja* ("Jhallas" or "Khar") ; *Saccharum spontaneum* ("Khas").

It was observed that the cercariae readily encysted on the leaves of *C. rotundus*, *I. reptans* and *C. dactylon*, which are smooth succulent grasses and are good food for animals acting as definitive hosts of the parasite. The cecariae were also found to encyst on the leaves of *H. aristata*, *C. bengalensis* and *H. vulgare*, but very reluctantly and when any of the first three named grasses was available they showed a preference for it. No encystment was noticed on the other grasses listed above. Their leaves have either sharp hairy spines on one or both the surfaces, or their edges are sharp and spiny. Animals, particularly sheep and goats, do not usually feed on these grasses. This selectivity on the part of the cercariae seems to afford evidence of a survival characteristic.

The swimming cercariae were observed to settle down at first on the grass blades, attaching their body to it firmly. They then secreted cystogenous material to form the cyst wall. Simultaneously the tail moved vigorously until it was finally shed. The cercariae were always found to encyst in groups or colonies and thus exhibited a gregarious "instinct".

Pathogenicity

Several workers (Baldrey, 1906 ; LeRoux, 1930 ; Pande, 1935 ; Mudaliar, *loc. cit.*, D'Souza, 1948 and Ramkrishnan, 1951) have recorded their observations on outbreaks of amphistomiasis in sheep, goats and cattle. Srivastava (*loc. cit.*) observed in experimentally infected kids that numerous immature amphistomes were found embedded in the wall of the duodenum and the initial part of the intestine, which were found to contain haemorrhagic fluid ; and the mucosa, specially of the duodenum, was markedly thickened and necrotic in places. The faeces were often found to be blood tinged and usually contained large numbers of immature amphistomes. Apart from these observations, no detailed account of the pathogenicity of this important parasite has been published so far.

Pure infections of *Cotylophoron cotylophorum* were established in clean lambs and a control lamb was maintained. Four lambs were given 996, 2,007, 3,000 and 4,996 metacercariae of this parasite and kept under close observation along with the control, on an infection-free diet. After 112 days the lamb which was given 3,000 metacercariae was destroyed to study the course of the infection and its pathology. A number of amphistomes, still sexually immature, were found in the rumen and reticulum. The abomasum contained semi-solid ingesta. The pylorus and anterior intestine, particularly the

duodenal portion, were found inflamed and slightly gelatinised. Several immature amphistomes were found embedded in the mucosa and were identified as *C. cotylophorum*.

The other lamb, which was given 4,996 metacercariae, started passing loose foetid stools from the 116th day after the infection. This was accompanied by progressive weakness and emaciation. Immature forms of *C. cotylophorum* were detected in the faeces after careful searching. Later, sub-maxillary dropsical swelling developed and the animal, which never had any rise of temperature, showed sub-normal temperature for 2 days prior to death which occurred on the 124th day after receiving the infection. On post-mortem examination the carcase was found in an extreme anaemic and cachexic condition. Subcutaneous, mesenteric and coronary fats were found gelatinised. Rumen and reticulum contained in all 58 sexually immature amphistomes, which were later identified as *C. cotylophorum*. The abomasum contained semi-liquid ingesta and the mucous membrane was found congested and partially gelatinised. The small intestine, particularly the duodenal portion about 3 ft. from the pylorus, was highly congested and the pyloric region was slightly gelatinised. In all, 1756 very small young immature amphistomes identifiable with *C. cotylophorum* were recovered from both the abomasum and the duodenum.

The other lambs, which were given 996 and 2,007 metacercariae respectively, and the control which received none, did not show any clinical symptoms for 180 days, when they were killed and examined. 168 and 362 young amphistomes developing to the sexually adult stage were recovered respectively from the rumen and reticulum of the infected lambs. These were identified as *C. cotylophorum*. No appreciable lesion was observed either in the abomasum or the duodenum. In the control, no amphistomes were found. All these three animals were in good condition throughout the experiment.

Histopathology. Infected portions of the duodenum were examined for histopathological changes. In Plate I, Figs. 1 to 3 show the parasites in different sites; when burrowing deeper into the submucosa they are surrounded by a zone of cellular infiltration. The cells are predominantly those of the mononuclear wandering type and in places a few giant cells are also seen. The mucous layer is thickened and the submucosa swollen. Desquamation of the mucosa from the underlying layers and also of the submucosa from the muscular layer beneath are markedly visible.

Summary

Observations were made on the biology of *Cotylophoron cotylophum* in respect of the hatching of miracidia, infection of the intermediary mollusc, emergence and encystment of cercariae, and on the pathogenicity of the parasite. It was noted that miracidia of this parasite hatch in 15 days at a temperature of 28–30°C. At a lower or higher temperature than this hatching is delayed. When the miracidia are mature a sudden fall in temperature hastens their hatching. Darkness has no adverse effect on hatching. These miracidia show a marked attraction towards *Indoplanorbis exustus*, which are proven to be the intermediary molluscs of this amphistome. At room temperature, ranging between 21–24°C. during the day and 18–21°C. at night, sporocysts develop within the snails in about 24 hours and on the 4th day rediae are seen developing inside the sporocysts. On the 6th day rediae are seen independently with small cercariae developing inside. Emergence of cercariae from the snails was noted from the 26th day. The cercariae are markedly heliotropic and the maximum emergence was noticed between the hours 11 a.m. and 2 p.m. at temperatures of between 30° and 35°C. The cercariae have a preference for a slightly alkaline medium and there is profuse cercarial emergence and much motility between *p*H 7 and 9·5. The cercariae show some preference for encystment on smooth succulent grasses, which are favoured by the definitive hosts and they encyst in groups or colonies. This tendency may have some survival value.

The pathogenicity of the parasite in clean experimentally infected lambs is described. It was found that about five thousand metacercariae produce clinical symptoms in about three and a half months ; these last for about a week and terminate in death. Lower infective doses did not produce disease. The parasites were found embedded in the mucosa of the pyloric region and in the first 3 feet of the duodenum ; they also burrowed deeper into the submucosa setting up a severe inflammatory condition with desquamation of the tissues from the underlying structures.

References

Baldrey, F. S. H., 1906.—"Some problems in sheep diseases". *J. trop. vet. Sci.*, **1**, 387–409. (W.L. 11588).

Bennett, H. J., 1936.—"The life history of *Cotylophoron cotylophorum*, a trematode from ruminants". *Illinois biol. Monogr.*, **14**, i–119. (W.L. 9222).

Chatterji, R. C., 1931.—"Preliminary observations on the life history of an amphistome cercaria, *Cercariae indicae* XXVI Sewell, 1922." *Zool. Anz.*, **95,** 117–179. (W.L. 23833).

————, 1938,—"Annotated list of helminths recorded from domesticated animals of Burma. Part I. Trematoda." *Proc. nat. Acad. Sci. India*, **8,** 93-104. (W.L. 16820a).

D'Souza, B. A., 1948.—"Observations on the outbreaks in 1946-1947 of the so–called obscure sheep disease at the Livestock Research Station, Hosur." *Indian vet. J.*, **25,** 321–330. (W.L. 9959).

Krull, W. H., 1932.—"Studies on the life-history of *Cotylophoron cotylophorum* (Fischoeder, 1901), Stiles and Goldberger, 1910." *J. Parasit.*, **19,** 165–166. (W.L. 11428).

————, 1934,—"Life-history studies on *Cotylophoron cotylophorum* Fischoeder, 1901), Stiles and Goldberger, 1910." *Ibid.*, **20,** 173–180.

Kuppuswamy, P. B., 1946.—Final Report on the Scheme for elucidation of etiology of "Pitto" and "Gillar" in sheep and goats of the Indian Council of Agricultural Research.

LeRoux, P. L., 1930.—"A preliminary communication on the life cycle of *Cotylophoron cotylophorum* and its pathogenicity for sheep and cattle." *Rep. vet. Res. S. Afr.*, **16,** 243–253. (W.L. 18640).

Mudaliar, S. V., 1945.—"Fatal enteritis in goats due to immature amphistomes, probably *Cotylophoron cotylophorum*." *Indian J. vet. Sci.*, **15,** 54–56. (W.L. 9941h).

Pande, P. G., 1935.—"Acute amphistomiasis of cattle in Assam : a preliminary report." *Ibid.*, **5,** 364–375.

Ramkrishnan, M., 1951.—"An outbreak of acute amphistomiasis among cattle in Nellore District." *Indian vet. J.*, **27,** 267–272. (W.L.9959).

Sinha, B. B., 1950.—"Life-history of *Cotylophoron*, a trematode parasite from the rumen of cattle, goats and sheep." *Indian J. vet. Sci.*, **20,** 1–11. (W.L. 9941h).

Srivastava, H. D., 1938.—"A study on the life-history and pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901), Stiles and Goldberg, 1910 of Indian ruminants and a biological control to check the infestation." *Ibid.*, **8,** 381–385.

Thapar, G. S. and Tandon, R. S., 1952.—"On the life-history of liver-fluke, *Fasciola gigantica* Cobbold, 1855 in India." *Indian J. Helminth.*, **4,** 1–36. (W.L. 9939g).

————, 1953.—"Addendum to the 'Life history of liver-fluke, *Fasciola gigantica* Cobbold, 1855 in India'." *Ibid.*, **5,** 121.

Varma, A. K., 1955.—"A simple method of breeding fresh-water molluscs in the laboratory." *Indian J. med. Res.*, **43,** 165–167. (W.L. 9940).

————, 1957.—"On a collection of Paramphistomes from domesticated animals in Bihar." *Indian J. vet. Sci.*, **27,** 67–76. (W.L. 9941h).

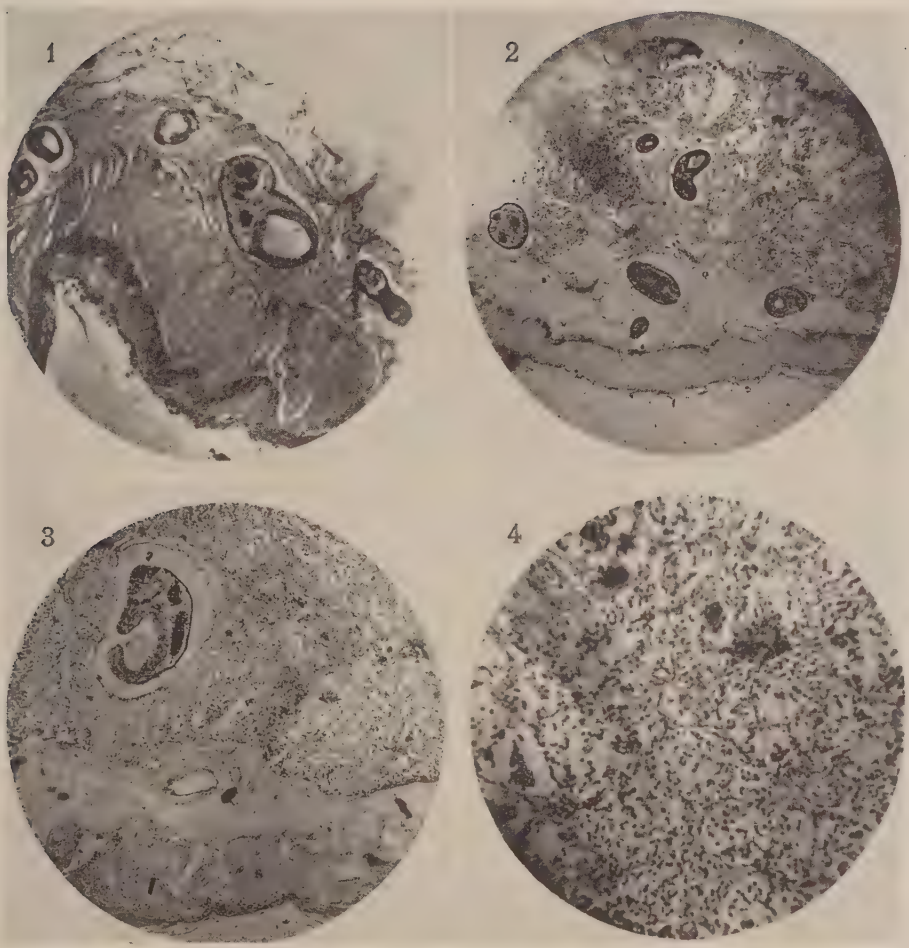


Plate I

FIG. 1.—Transverse section of an infected portion of duodenum showing young amphistomes embedded in the mucosa, which is thickened and desquamating (H.E.× 6). FIG. 2.—Transverse section of duodenum showing young amphistomes burrowing deeper into the subcutaneous layer, with visible inflammatory reaction. Note desquamation of the submucosa. (H.E.× 9). FIG. 3.—Young amphistome in submucosa, surrounded by cellular infiltration. (H.E.× 15). FIG. 4.—Zone of cellular infiltration showing mononuclear wandering cells and some giant cells. (H.E.× 50).

Journal of Helminthology, Vol. XXXV ,Nos. 1/2, 1961, pp. 169–180.

Systematic Studies on Some Cestodes of Reptiles and Birds*

By S. Wahid

From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

During systematic studies of helminths of reptiles and birds the writer found a very interesting cestode which was collected from *Agama cyanogaster* and is described here as *Megacapsula leiperi* gen. et sp. nov. Two other cestodes, *Oochoristica zonuri* Baylis, 1919, and *Arostellina reticulata* Neiland, 1955, are recorded from new hosts and are redescribed.

Anoplocephalidae Cholodkovsky, 1902

Linstowinae Fuhrmann, 1907

Megacapsula gen. nov.

Megacapsula leiperi n.sp.

One complete and some broken specimens, two with scolices, were available for study. The single complete specimen measures about 60 mm. in length and 1–27 mm. in maximum thickness. The strobila is narrow in the anterior part and the maximum width is found in the gravid segments. Most of the mature segments are broader than long, though some square segments are also present. The scolex is not separated from the strobila by any constriction. The suckers are oval in shape, deeply set and rather muscular. They measure 90–100$\mu \times 135\mu$. The diameter of the scolex is 240–400μ. Conspicuous segmentation starts at about 1·8 mm. from the posterior end of the suckers. The mature segments are 0·3–0·33 mm. long and 0·63–0·84 mm. wide. The gravid segments measure 0·97–1·27 mm. in length and 1·1–1·2 mm. in width.

The genital pores alternate irregularly and open in the anterior third of the segment's margin. The rudiments of the genital organs appear at about 1·8 mm. from the scolex.

The ovary is situated in the middle of the segment or slightly poral in position. It is clearly divided into two lobes connected by a narrow part and measures 0·15–0·21 mm. across the segment. The

* Part of a thesis approved by the University of London for the award of the M.Sc. degree.

vitelline gland is irregular in shape, measuring 0·075–0·1 mm. and it is situated posterior to the ovary. A small ootype is present between the ovary and the vitelline gland. The vagina is very thin and opens posterior to the cirrus pouch. A small receptaculum seminis is present.

The testes are circular in outline and are situated posterior to the female glands. There are 26–30 testes and they measure 21–23μ in diameter. The vas deferens is convoluted. No seminal vesicle is present. The unarmed cirrus sac is 105–120μ in length and extends to the excretory vessels.

The uterus appears in the anterior part of the segment as a transverse sac which finally breaks up into capsules. These capsules are very conspicuous and large, each containing one to ten eggs. (Fig. 4). The eggs measure 0·037–0·041 mm. and the onchospheres are 0·03–0·034 mm.

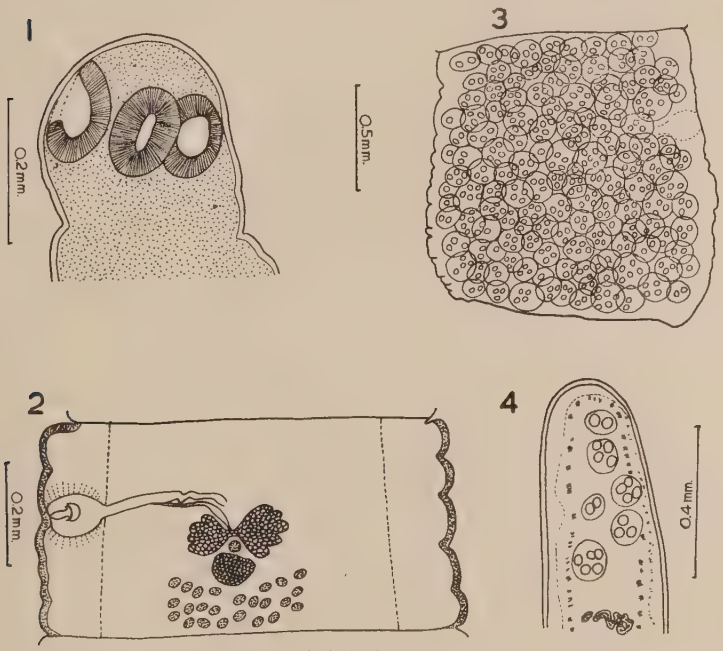
Relationships : The specimens described above resemble the genus *Oochoristica* Lühe, 1898, in the shape of the scolex and the arrangement of the genital organs. All the species of the genus *Oochoristica* described up to the present time possess a uterus which breaks up into egg capsules each containing a single egg. The present specimen differs from *Oochoristica* in possessing a uterus which breaks up into egg capsules each containing several eggs.

The other genera of the subfamily Linstowinae Fuhrmann, 1907, recorded from reptiles are *Pancerina* Fuhrmann, 1899, and *Diochetos* Harwood, 1932. The genus *Pancerina* contains a double set of genital organs and the genus *Diochetos* is very close to *Oochoristica* in almost all respects and is not considered a valid one. In the same subfamily the genera *Inermicapsifer* Janicki, 1910, and *Thysanotaenia* Beddard, 1911, possess a uterus which breaks into egg capsules, each capsule containing several eggs. But the present specimen differs from them in possessing irregularly alternating genital pores ; in the above two genera the genital pores are unilateral and they are recorded from mammals.

The character of the uterus is an important factor and is used in the taxonomy of different groups of cestodes. The genera *Choanotaenia* Railliet, 1896, and *Paricterotaenia* Fuhrmann, 1932, are separated on the basis of the type of uterus they possess. The genus *Raillietina* Fuhrmann, 1920, is divided into subgenera mainly on the character of the uterus which contains one or more eggs in each capsule.

Considering the difference discussed above the writer considers it advisable to erect a new genus *Megacapsula*, in the subfamily

Linstowinae, for the reception of these specimens. For this new cestode the name *Megacapsula leiperi* is proposed in honour of Professor R. T. Leiper.



Megacapsula leiperi gen.et sp.nov.

1.—Scolex. 2.—Mature segment. 3.—Gravid segment. 4.—Transverse section (in part) of a gravid segment.

Megacapsula gen. nov.

Generic diagnosis : Linstowinae ; medium-sized worms. Genital pores alternating irregularly. Genital ducts pass between the osmoregulatory canals. Testes numerous. Gravid uterus breaking down to egg capsules each containing several eggs. Parasites of reptiles.

Host : *Agama cyanogaster*

Location : Intestine

Locality : Rhodesia

Oochoristica Lühe, 1898

The genus *Oochoristica* was erected by Lühe (1898) to accommodate many species of cestodes from reptiles. Later it was also reported from Carnivores, Insectivores, Edentates and South American Marsupials.

It is a large genus with a very wide geographical distribution. It was reviewed by Hughes (1940), Lopez-Neyra (1954) and Dollfus (1954) who gave a comprehensive table of the specific characters of species of *Oochoristica* found in the reptiles of the Old World.

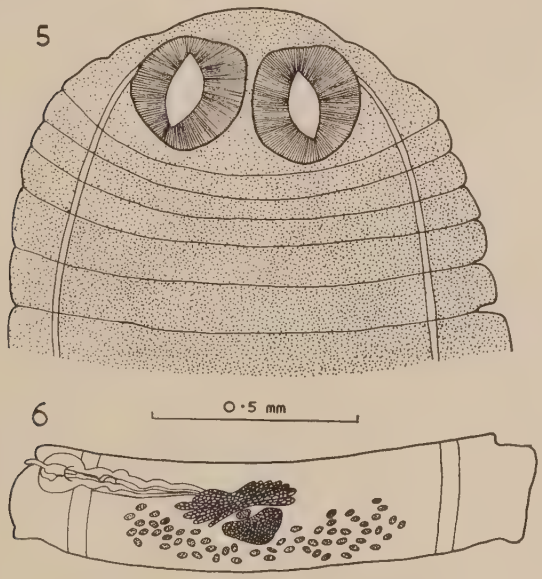
Dollfus (1954) also described three new species, *O. gallica*, *O. chabaudi*, *O. salensis* and a new variety *O. gallica* var. *pleionorcheis*. Della Santa (1956) recognised 57 valid species of the genus of which 39 are described from reptiles. Lopez-Neyra and Diaz-Ungria (1957) listed (44) forty-four species (with two varieties) from reptiles. In the same paper they described a new species *O. insulaemargaritae* from *Ameiva ameiva*. Another species *O. tandani* was later added by K. S. Singh (1957), from a snake *Lycodon aulicus* in India. In the same year Alexander, C. G. and Alexander, E. P. described *O. crotalicola* from California rattlesnakes and Dollfus (1957) added *O. darensis*, *O. pseudocotylea* and a new variety of *O. rostellata* Zschokke, 1905, namely *O. rostellata* var. *agamicola*.

Oochoristica zonuri Baylis, 1919

This species is redescribed here from a new host, *Psammophis sibilans*. The scolex is relatively large and measures 750–800μ across the suckers. The suckers are 270–300μ in diameter. The strobila measures up to 40 mm. in length. The anterior part of the strobila is wider than the posterior part. One typical mature segment measures $0{\cdot}25 \times 1{\cdot}26$ mm. in the length and width respectively. The maximum width of the strobila is 1·7 mm.

The genital pores alternate irregularly and open in the anterior half of the margin of the segment. The genital ducts pass between the excretory vessels. The ovary is slightly bilobed, each lobe containing small lobules, and measures 0·23–0·35 mm. across. The vitelline gland is compact, somewhat triangular in shape and situated posterior to the ovary, measuring 0·12–0·15 mm. A small ootype is present between the ovary and the vitelline gland. The vagina opens posterior to the cirrus pouch. Just before reaching the ovary it dilates to form a small receptaculum seminis.

There are 55–72 testes, situated posterior and lateral to the female glands. They measure 0·04–0·056 × 0·018–0·026 mm. and are ellipsoid in shape. The vas deferens is convoluted; the cirrus pouch reaches the excretory vessels and measures 165–180 mm. in length. It is unarmed.



Oochoristica zonuri Baylis, 1919
5.—Scolex. 6.—Mature segment.

The uterus finally breaks up into egg capsules each containing a single spherical egg which measures 0·037–0·04 mm. The onchospheres are 0·034 × 0·026 mm. with long, prominent embryonic hooks.

The specimens described herein closely resemble *O. zonuri* Baylis, 1919, in possessing a large scolex and suckers, in the number of testes, and in other important measurements. *O. zonuri* was originally described by Baylis (1919) from *Zonurus tropidosternum* in Africa. The other species of *Oochoristica* recorded from *Psammophis*

sibilans are *O. agamae* Baylis, 1919, *O. tuberculata* (Rudolphi, 1819) and *O. ameivae* (Beddard, 1914). *O. zonuri* differs from them mainly in the number of testes.

Host : *Psammophis sibilans* (new host)

Location : Intestine

Locality : Rhodesia

DILEPIDIDAE Railliet and Henry, 1909

DILEPIDINAE Fuhrmann, 1907

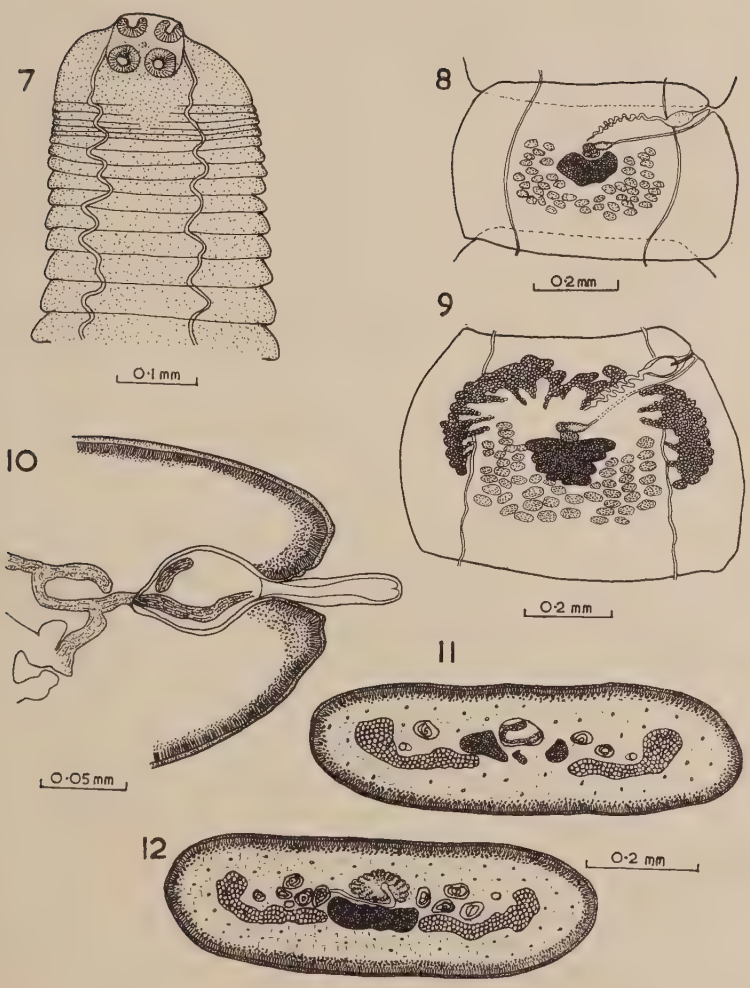
Arostellina Neiland, 1955

Arostellina reticulata Neiland, 1955

A few specimens with scolex as well as some fragments of mature and semi-gravid segments were available for study. No gravid segments were present. The segments are broader than long except for a few of the very last segments, 2 or 3 in number, which are squarish or slightly longer than broad. The length of the strobila is 7–9·45 mm. and the maximum width 0·87 mm. The scolex is flattened dorsoventrally. The four suckers are deeply set, 0·5–0·52 mm. in diameter, rather feebly developed, rounded and unarmed. In a specimen 7·2 mm. long the scolex measures 0·12 mm. across the suckers. The scolex is followed by a short neck about 0·075 mm. long. This shortness is due to the contracted condition of the specimens.

The genital pores alternate irregularly and open in the anterior fourth of the border of the segment in a deeply situated genital atrium. The genital organs first appear in segments number 20–22. The ovary occupies the central part of the segment and measures 0·18–0·225 × 0·12–0·15 mm. It is compact and undivided; its anterior part is wider than the posterior and this gives it a triangular appearance in the later segments. The vitelline gland, situated anterior to the ovary and the ootype, is very small. The vagina opens in the genital atrium, posterior to the cirrus. A prominent receptaculum seminis is present. The size of the receptaculum seminis enlarges as the segments grow older (mature) and measures 0·075–0·15 × 0·045–0·068 mm.

The testes are numerous and irregularly oblong in shape. They are distributed posterior and lateral to the female organs in a horseshoe shape. They vary in number between 68–73 and measure



Arostellina reticulata Neiland, 1955

7.—Scolex. 8.—Mature segment. 9.—Semi-gravid segment. 10.—Section through the cirrus sac. 11 and 12.—Transverse sections of a semi-gravid segment.

0·056–0·064 × 0·026–0·04 mm. The vas deferens is very much coiled and is surrounded by conspicuous small cells. No external or internal seminal vesicle was observed. The cirrus opens anterior to the vagina and is enclosed in a pear-shaped cirrus sac measuring 0·07–0·086 mm. in length.

As mentioned above no gravid segments were present so a detailed description of the uterus cannot be given. It starts from the ventral surface and gradually develops towards the dorsal surface to occupy the anterior part of the segment. Laterally some follicular structures fill the segments outside the excretory vessels (Fig. 9). These follicles are inconspicuously connected with the developing branches of the reticulate uterus. The connection of these follicles and the uterus is clarified to some extent in the transverse sections of the segments (Figs. 11 and 12).

Relationships : The specimens described here fall in the genus *Arostellina* Neiland, 1955, because of the absence of a rostellum ; in having four feebly developed unarmed suckers ; in the position of the vitelline gland which is anterior to the ovary, and in possessing a reticulate uterus.

In Table I the measurements of *A. reticulata* from the original description and that of the present material are given. Except for the length of the strobila the general measurements are very close. In the specimens described by Neiland (1955) the strobila is 27 mm. long ; the low maximum length here is probably due to the absence of fully grown specimens with gravid segments and to their contracted condition. As these differences are not considerable the specimens are regarded as belonging to *A. reticulata* Neiland, 1955.

Yamaguti (1960), transferred *Arostellina* to the family Anoplocephalidae Cholodkovsky, 1902, in the subfamily Anoplocephalinae Blanchard, 1891. No reasons for the assignment are given. Neiland (1955) placed *Arostellina* in the family Dilepididae Railliet and Henry, 1909, on the basis of the following characters.

1. The presence of numerous testes.
2. The absence of seminal vesicle.
3. Alternating genital pores.
4. Reticulate uterus.
5. Lack of paruterine organs.
6. Eggs not encapsulated.

Most of the members of the subfamily Dilepidinae possess a retractable rostellum which is commonly armed with one or more circles of hooks. *Arostellina* is devoid of any rostellum. The only other genus without rostellum in this subfamily is *Cotylorhipis* Blanchard, 1909, which possesses armed suckers. It also differs from Dilepidids in having the vitelline gland anterior to the ovary.

The other genera of the family having a reticulate uterus are *Similuncinus* Johnston, 1909, *Dendrouterina* Fuhrmann, 1912, *Cladogynia* Baer, 1937, *Pseudandrya* Fuhrmann, 1943, *Dictymetra* Clark, 1952, and *Lapwingia* Singh, 1952, but the above genera possess a rostellum.

The vitelline gland is found in front or partly behind the ovary in the genera of the family Tetrabothriidae Linton, 1891 ; but it differs from Tetrabothriids in the presence of a reticulate uterus and the irregularly alternating genital pores.

Host : *Clytolaema rubricauda* (Brazilian ruby humming bird). New host.

Location : Small intestine
(Collected at London Zoo)

TABLE I

	Arostellina reticulata Neiland's measurements	*A. reticulata* Present specimens
Length of the strobila	27 mm.	7·2–9·4 mm.
Maximum width	0·85 mm.	0·87 mm.
Diameter of the scolex	—	0·12 mm.
Diameter of the suckers	0·07–0·08 mm.	0·05–0·052 mm.
Length of the cirrus sac	0·11–0·13 mm.	0·07–0·086 mm.
Number of testes	60–76	68–73.
Size of testes	0·045–0·075 × 0·040–0·075 mm.	0·056–0·064 × 0·026–0·04 mm.
Ovary	0·19–0·25 mm. long 0·18–0·18 mm. broad	0·12–0·15 mm.
Receptaculum seminis	0·8–0·18 ? (0·08–0·18)	0·075–0·150 long × 0·045–0·068 wide
Eggs	0·020–0·025 in dia.	—

Dipylidiinae Stiles, 1896

Choanotaenia Railliet, 1829

Kowalewskiella hypoleucia (Singh, 1952) n.comb.

Syn. *Choanotaenia hypoleucia* Singh, 1952

Singh, K. S. (1952) first described this species from *Tringa hypoleucos* (Charadriiformes) and assigned it to the genus *Choanotaenia*.

Ch. hypoleucia is characterised by having 26 rostellar hooks in a single row measuring 0·006–0·007 mm. in length. The testes are 35–50, anterior and posterior to the female glands. The mature segments are considerably longer than broad.

Singh (1952) gave no reasons for assigning this species to the genus *Choanotaenia*. As far as can be judged from the description and figures given in the original description this species belongs to the genus *Kowalewskiella* Baczynska, 1914. The genus *Choanotaenia* and *Kowalewskiella* are close to each other differing only in the following two main characters.

1. The mature segments are longer than broad in *Kowalewskiella* and broader than long in *Choanotaenia*.

2. The testes are situated anterior and posterior to the female glands in the species of *Kowalewskiella* and only posterior or sometimes lateral to the female glands in the species of *Choanotaenia*.

The writer suggests that *Ch. hypoleucia* Singh, 1952, should be transferred to the genus *Kowalewskiella*.

Acknowledgments

I am very grateful to Professor J. J. C. Buckley for his supervision and advice during this work. I am also thankful to Mr. S. Prudhoe of the helminth section, British Museum (Nat. Hist.) for his helpful suggestions.

REFERENCES

ALEXANDER, C. G. and ALEXANDER, E. P., 1957.—"*Oochoristica crotalicola*, a new Anoplocephalid cestode from California rattlesnakes." *J. Parasit.*, **43,** 365–366. (W.L. 11428).

BAER, J. G., 1927.—"Monographie des cestodes de la famille de Anoplocephalides." *Bull. biol.*, France et Belgique, Suppl. **10,** 241 pp. (W.L. 3919).

BAYLIS, H. A., 1919.—"On two new species of the cestode genus *Oochoristica* from lizards." *Parasitology*, **11,** 405–414. (W.L. 16035).

CLARK, D. T., 1952.—"Three new dilepidid cestodes, *Dictymetra numenii* n.gen., n.sp., *Dictymetra paranumenii* n.sp., and *Anomotaenia filevata* n.sp." *Proc. helm. Soc. Wash.*, **19,** 18–27. (W.L. 16747a).

DELLA SANTA, N., 1956.—"Révision du genre *Oochoristica* Lühe (Cestodes)." *Rev. suisse Zool.*, **53,** 1–113. (W.L. 19288).

DOLLFUS, R. Ph., 1954.—"Miscellanea Helminthologica Maroccana XVIII. Quelques cestodes dur groupe *Oochoristica* auctorum recoltes au Maroc, avec une liste de cestodes de hérissons (Erinaceidae) et une liste du Sauriens et Ophidiens (exclus. Amèrique et Australie) ou ont été trouvés de *Oochoristica*." *Arch. Inst. Pasteur Maroc.*, **4,** 657–711. (W.L. 1842e).

————, 1957.—"Miscellanea Helminthologica Maroccana. XIX Nouvelles récoltés d'Oochoristica chez des Sauriens du Maroc." *Arch. Inst. Pasteur Maroc.* **5,** 272–299. (W.L. 1842e).

FUHRMANN, O., 1932.—"Les Tenias des Oiseaux." *Mem. Univ. Neuchâtel*, **8,** 381 pp. (W.L. 13345).

HUGHES, R. C., 1940.—"The genus *Oochoristica* Lühe, 1898." *Amer. Midl., Nat.*, **23,** 368–381. (W.L. 640).

KINTNER, K. E., 1938.—"Notes on the cestodes of English sparrows in Indiana." *Parasitology*, **30,** 347–357. (W.L. 16035).

LINCICOME, D. R., 1939.—"A new tapeworm, *Choanotaenia iola*, from the robin." *J. Parasit.*, **25,** 203–206. (W.L. 11428).

LOPEZ-NEYRA, C. R., 1951.—"Analises critica de los generos *Choanotaenia*, *Anomotaenia* y afines con redescripcion de la *Taenia porosa* Rudolphi, 1819 (1810) e invalidez del genera *Paricterotaenia* Fuhrmann, 1932. (Prima parte)." *Rev. iber. Parasit.*, **11,** 337–368. (W.L. 18858c).

————, 1952.—idem. (Second part and conclusion). *Ibid.*, **12,** 1–58.

————, 1954.—"Anoplocephalidae." *Ibid.*, **14,** 13–130, 225–290, 303–396.

LOPEZ-NEYRA, C. R. and DIAZ-UNGRIA, C., 1957.—"Cestodes de Venezuela III. Sobre unos cestodes intestinales de reptiles y mamiferos. venezolanos." *Mem. Soc. Cienc. nat. La Salle*, **17,** 28–63. (W.L. 13480a).

NEILAND, K. A., 1955.—"The helminth fauna of Nicaragua. 1. A new genus and species of cestode (Dilepidinae) from the humming bird, *Phaeocheroa cuvierii roberti*." *J. Parasit.*, **41,** 495–498. (W.L. 11428).

SINGH, K. S., 1952.—"Cestode parasites of birds." *Indian J. Helminth.*, **4,** 1–72. (W.L. 9939g).

————, 1957.—"*Oochoristica tandani* n.sp. (Cestoda) from a snake, *Lycodon aulicus*, from India." *J. Parasit.*, **43,** 377–379. (W.L. 11428).

SOUTHWELL, T., 1930.—The fauna of British India, including Ceylon and Burma. Cestoda II. 262 pp.

SPASSKI, A. A., 1951.—Principles of cestodology, edited by K. I. Skryabin. Vol. 1. "Anoplocephalata—tapeworms of domestic and wild animals." Moscow : Izdatelstvo Akademii Nauk U.S.S.R. 735 pp. (in Russian) (Reference not seen).

Stunkard, H. W. and Milford, J. J., 1937.—"Notes on the cestodes of North American sparrows." *Zoologica*, New York, **22**, 171–183. (W.L. 23821).

Wardle, R. A. and McLeod, J. A., 1951.—The Zoology of Tapeworms. Univ. Minnesota Press.

Yamaguti, S., 1959.—Systema Helminthum, Vol. 2. The Cestodes of Vertebrates. 860 pp. Interscience Publishers.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, pp. 181–202.

The Dimorphism of *Polystoma integerrimum* (Frölich) Rudolphi and Its Bearing on Relationships within the Polystomatidae: Part III*

By Joan B. Williams, M.Sc., Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

Discussion

Several discrepancies are to be found in the literature on *Polystoma* and related genera, the most significant of these concerning the anatomy of the neotenic form of *Polystoma integerrimum*. Zeller (1872, 1876) mistook the relations of the genito-intestinal canal, believing it to be an internal vas deferens or sperm conducting canal extending from the testis to the junction of the oviduct and common vitello-vaginal canal. He believed that internal self insemination occurred by way of this duct in all neotenic adults and in solitary normal individuals. Zeller considered that self insemination in single bladder adults could be either internal through this canal or external through the vaginae. Ijima (1884) showed that the "internal vas deferens" of the normal form communicates not with the testis but with the intestine, and Goto (1891) subsequently named it the genito-intestinal canal. With the discovery of the true relations of the genito-intestinal canal, the assumption that internal self insemination occurs in the normal adult became untenable.

Zeller observed the vas deferens and copulation complex of the gill adult, but thought them to be functionless and considered insemination to be exclusively internal.

Gallien (1935) denied the existence in the neotenic form of both vas deferens and genito-intestinal canal, and described an internal sperm duct from the testis communicating with the junction of the oviduct and common vitelline duct. He regarded the copulation apparatus of the neotenic adult as a non-functional vestige. Gallien, like Zeller, believed internal self insemination to occur in the branchial adult, and considered cross fertilization and external self insemination to be impossible (1935, p. 39).

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

According to Paul (1938) the neotenic adult of *Polystoma nearcticum* (formerly *Polystoma integerrimum* var. *nearcticum*) lacks a genito-intestinal canal and possesses a rudimentary penis, and Paul believed that internal self insemination may occur in this form. He also stated : "... in agreement with Odhner's classification of the Monogenea which emphasized the presence or absence of a genito-intestinal canal, it has been considered that the monogenetic sub-orders constitute two naturally distinct groups. . . . However, in the same genus, and moreover, in the same species, *Polystoma integerrimum*, there is a bladder form with a genito-intestinal canal and a branchial form without one. This situation may invalidate the present concept of origin and relationship." However, reinvestigation of the branchial adult of the American form may disclose a genital system similar to that of the neotenic form of the European species.

In the gill form of *Polystoma integerrimum* insemination must take place by way of the ovo-vitelline duct ; internal self insemination is impossible. External self insemination presumably occurs in solitary neotenic worms, and when two or more adult individuals inhabit the branchial chamber of a tadpole, copulation by way of the gonopore is probably feasible. Presumably cross insemination is effected via the ovo-vitelline duct in *Protopolystoma* and apparently also in *Sphyranura*. Thus, while in the normal form of *Polystoma* self- and cross-insemination occur exclusively through the paired copulation canals, in the branchial form presumably both self- and cross-insemination take place solely by way of the ovo-vitelline duct.

The vas deferens of the normal form is developed at a comparatively early stage, being visible at the end of four months (Zeller, 1872). The penial spines are not secreted until after the formation of both vas deferens and genito-intestinal canal (the "internal vas deferens"). Thus, the normal form passes through a phase of development at which it resembles the neotenic adult.

Hence comparatively slight genetic changes, causing the rates of the complex, interrelated processes of development to be readily influenced by environmental factors, may have given rise to the duality of the adult form. The evolution of an internal canal uniting male and female organs in the neotenic form, as is implied by Gallien's account, would involve major changes in genetic constitution.

It is not clear from the available data whether the gonopore during normal development initially communicates with the oviducal chamber, as is permanently so in the neotenic adult, or

opens only after the uterus is formed. The former possibility would presumably entail a forward displacement of the gonopore and penis bulb as the uterus develops, with corresponding elongation of the vas deferens.

Both the development of the body and the maturation of the gonads are accelerated in the neotenic form ; however, the gonads mature more rapidly in relation to the general ontogeny than occurs during the normal life history, and somatic development ceases prematurely. It is not simply lack of time which prevents the completion of development, for Gallien (1935) finds that the neotenic animals eventually become senescent, and die irrespective of the metamorphosis of the tadpole.

It is interesting to note that, within the one species, the copulatory function normally subserved by the paired copulation canals is adopted by the ovo-vitelline duct as a consequence of reduction resulting from neotenic development.

Further discrepancies in the literature are involved in the description of the ovo-vitelline duct, particularly in the identification of the ootype. Thus Wright and MacCallum (1887) considered the junction of the left and right vitelline ducts together with the junction of the common vitelline duct and oviduct to constitute the ootype in *Sphyranura osleri*. They state (p. 42) :

> "The ootype is here formed of two distinct parts, one which receives the ducts from the receptacula seminis, and another in which centre the ovarian and uterine tracts of the oviduct, as well as the overflow tube of the intestine. The latter part hardly affords accommodation for more than the large ovarian ovum, so we gather that the arrangement of the food-yolk balls around the egg takes place in the uterus."

As previously mentioned, *Sphyranura* lacks a uterus, the "uterus" of Wright and MacCallum clearly being the ootype. These authors write (pp. 44, 45) :

> "The uterine tract is folded, and undergoes a marked constriction before it passes into the uterus. This organ is imbedded in a mass of unicellular glands, a great many of which empty their contents into the oviduct at the above-mentioned constriction, while others pierce the dorsal and ventral wall of the uterus, towards which their ducts converge."

Similarly, Alvey (1936) considered the junction of the common vitelline duct and oviduct of *Sphyranura oligorchis* to represent the ootype, and identified the ootype as uterus. Stunkard's (1917) account of *Polystomoides* and related genera contains similar inaccuracies, and other authors have followed these misconceptions.

It seems that where a Mehlis' gland associated with the "ootype" of these writers was identified, nerve cells in the plexus surrounding the junction of the common vitello-vaginal canal and the oviduct may in fact have been observed.

The term uterine duct as used by the above-mentioned authors applies to the narrow proximal part of the ovovitelline duct. This term was proposed by Wright and MacCallum (1887) and Stunkard (1917) for the duct believed to be interposed between ootype and uterus, by analogy with the female duct system of cestodes. However, because it was based on a misconception this name should be discarded. The descending limb of the uterus in *Diplorchis* and *Parapolystoma,* which lies between the ootype and the wide ascending limb of the uterus, does not correspond with the so-called uterine duct of other genera (c.f. Ozaki, 1935).

Protopolystoma, while having essentially the same structure as the branchial form of *Polystoma,* has the same location in the host as the normal form of this genus. *Protopolystoma* may have evolved from a stock possessing a uterus and paired copulation canals and resembling the normal adult of *Polystoma,* and may be a permanently neotenic form, rather than primitively simple in structure as suggested by Bychovsky (1957). (The generic name suggested by Bychovsky may therefore be misleading.) The occurrence of a penial coronet in *Protopolystoma* perhaps indicates that the copulation canals have been secondarily lost in this genus. The large spines in the penial coronet of *Protopolystoma* closely resemble the penial spines of *Polystoma* : the ends of the spines are blunt and diverging, and the coronet may function in the fashion of a speculum, facilitating the insertion of the penis, as is known to occur in *Polystoma integerrimum.* However, stimulus for the evolution of the elaborate coronet was probably provided by the concomitant development of minute pores such as occur in the vaginal prominences of *Polystoma, Parapolystoma* and *Diplorchis* rather than by the relatively wide gonopore, through which impregnation must occur in *Protopolystoma.*

In *Polystomoides* and related genera the copulation canals open by minute pores, but possess large funnel-shaped openings.

The canals are short and their openings are at a relatively posterior level. The penial coronet of these genera resembles that of *Polystoma*. Wide funnel-like vaginal apertures presumably would not have provided sufficient stimulus for the evolutionary development of the coronet, and similarly in these genera the copulation canals may have undergone reduction or modification.

It seems that the vaginae of *Sphyranura* are also modified. Wright and MacCallum (1887) believed that the vaginae of *Sphyranura osleri* have undergone reduction and lost their surface connection, the vestigial ducts undergoing a concomitant change in function and serving as seminal receptacles for sperms introduced through the ovovitelline duct. Alvey (1936) states that the vaginae of *S. oligorchis* are vestigial and terminate in solid enlargements. It therefore appears that the copulation canals of *Sphyranura* are secondarily reduced, and the well-formed penial coronet of this genus may indicate the previous occurrence of minute copulation pores and small terminal vaginal canals.

Hence it seems that vaginae with terminal prominences traversed by small canals, such as are found in the genera *Polystoma*, *Parapolystoma* and *Diplorchis*, were primitively present in the Polystomatidae. It is noteworthy that these genera are characterized by the possession of a long winding uterus, and all parasitize anuran hosts.

Zeller (1876) drew attention to the fact that *Polystomoides* (then *Polystoma*) *ocellatum* resembles the neotenic form of *Polystoma* in lacking a uterus. Later de Beer (1940) suggested that the absence of a uterus in *Polystomoides* was the outcome of a neotenic evolutionary change. As remarked previously, a uterus is absent also in *Sphyranura*.

The caudal suckers of *Polystomoides* and similar genera are elaborately cuticularized and divided into annular zones, and suckers of a similar kind are possessed by *Oculotrema*. Throughout the Monogenea is shown a progressive tendency towards elaboration of the opisthaptor and cuticularization of the suckers, and in this respect these genera show an evolutionary advance beyond the condition in the parasites of Anura. Also, it may be indicative of further evolutionary progress that *Polystomoides* and related genera and *Oculotrema* parasitize hosts of higher systematic position than do the genera having poorly cuticularized, undivided caudal suckers.

From the foregoing indications it seems that the simplification of the genital system met with in all polystomatid genera other than *Polystoma*, *Parapolystoma* and *Diplorchis* is secondary rather than

primitive, and the occurrence of dimorphism in *Polystoma* lends indirect support to this view.

In *Sphyranura*, additional features may be interpreted as either primitive characters or the outcome of evolutionary rejuvenation : the caudal disc bears a single pair of suckers only ; the larval hooklets persist throughout life, whereas the hooklets are generally lost in the adult stages of other genera ; and the animals are agile and move about on the host in a lively manner. Further, *Sphyranura* has a very short life span, the life cycle being completed in less than two months in the laboratory (Alvey, 1936). This contrasts strikingly with the life span of six years or so of the normal form of *Polystoma integerrimum*, but is comparable with the two to three months' length of life of the branchial form. The brief life history of *Sphyranura* could be either an unmodified primitive feature or the result of neotenic evolution.

Wright and MacCallum (1887) considered the single pair of suckers of *Sphyranura* to signify that this genus occupies a position intermediate between *Gyrodactylus* and *Polystoma*. However, as remarked earlier, the vaginae are apparently secondarily reduced in *Sphyranura* ; it seems that their communication with the exterior is lost, yet a well formed penial coronet remains. The caudal suckers are well developed and divided into zones by cuticular partitions, as occurs also in the genera parasitic on reptiles and mammals. The energetic movement shown by *Sphyranura* is atypical among flukes with a posterior attachment disc bearing highly evolved suckers. Also, the development of *Sphyranura* is secondarily modified, the gyrodactyloid larval stage characteristic of the Polystomatidae having been eliminated from the life history. From these indications taken together it seems that the simplified or larval characters of this genus are more probably due to secondary reduction or evolutionary rejuvenation than to primitivity.

Thus it may be that in *Sphyranura* the development of the opisthaptor is permanently arrested as a result of a neotenic evolutionary change. The caudal suckers of *Polystoma* arise in pairs, and a young *Polystoma* at the two-sucker stage is strongly reminiscent of the adult *Sphyranura*. It is interesting also to note that the neotenic form of *Polystoma integerrimum* at times attains maturity and begins to lay eggs before all the caudal suckers are fully developed.

Hence it appears that a general tendency towards neotenic development, principally affecting the development of the female genital ducts, may be shown throughout the group. Neoteny has

apparently occurred along several independent lines of evolution : thus in the branchial forms of the dimorphic species parasitic in terrestrial Anura and in *Protopolystoma* both uterus and copulation canals are lost ; in the parasites of aquatic reptiles the uterus is absent and the terminal portions of the vaginae simplified ; possibly copulation canals are secondarily wanting in *Oculotrema* ; while *Sphyranura* lacks a uterus, evidently possesses reduced vaginae, shows considerable agility, and has a single pair of caudal suckers and persistent larval hooklets.

A further interesting feature of *Sphyranura* is that the newly hatched worm, though unciliated, is able to swim by movements of the caudal disc (Alvey, 1933 ; 1936), and it seems that the capacity for free-swimming is retained by the adult (Wright and MacCallum, 1887).

It seems also that neoteny may have influenced the proportions of the various parts of the body. Gallien (1935) drew attention to the following manifestations of heterogenic growth (allometry) in the normal form of *Polystoma integerrimum* : as the size of the worm increases, the relative size of the pharynx and the anchors decreases, and the eyes and intestinal bifurcation become relatively more anteriorly placed. He pointed out that these heterogenic growth phenomena would be consistent with an area of maximum growth in the middle of the body, i.e. that a growth gradient or pattern exists which has a peak of maximum intensity in this position. Gallien observed that similar heterogenic changes occur during the growth of the neotenic individuals. He noted also that the body of the larvae is comparatively more slender than that of the adult.

Thus, in correlation with a region of maximum growth intensity in the middle of the body, the various organs become relatively smaller and displaced towards the ends as the worm ages. The body proportions of neotenic adults more closely resemble the proportions of young normally-developing animals than those of older normal adults. However, the transverse vitelline ducts of the neotenic adult are comparatively further posterior in position than the vitello-vaginal canals of a young normal animal of equal length ; in the former the ducts approximately bisect the body, while in the latter they lie about one third the length of the body from the anterior end. The transverse ducts of the normal and neotenic forms of *Polystoma nearcticum* also differ in relative position, although in this species the normal and neotenic adults show a comparable variation in size (Paul, 1938).

Hence, although the heterogenic growth changes are basically the same in the neotenic as in the normal form, they relate to a

different scale or follow a slightly dissimilar pattern.

Although the available data are scanty, it is apparent that there is wide variation in relative proportions of the parts of the body among members of the Polystomatidae. It seems that the proportions of the various parts of the body in the genera which do not parasitize terrestrial amphibia resemble more closely the proportions of the neotenic form or the larva of *Polystoma* than those of the normal form of *Polystoma, Parapolystoma,* or *Diplorchis.*

As is characteristic of the branchial form of *Polystoma,* the transverse female ducts (vitelline ducts, or vitello-vaginal canals if copulation canals are present) occupy a relatively posterior level in the genera *Polystomoides, Neoplystoma, Polystomoidella* and *Protoplystoma.* By contrast, these ducts tend to be situated near the anterior end in the genera parasitic in terrestrial amphibia, with the exception of *Polystoma cachani* Gallien, in which uterus and vaginae are considerably elongated. [Compare, for example, the genera parasitizing turtle (Price, 1939) with the normal form of *Polystoma* (Gallien, 1935, Pl. 1, Fig. 1) and *Diplorchis* (Ozaki, 1935, Fig. 5)]. In *Polystomoidella oblongum* (Stunkard, 1916, Pl. 5, Fig. 31) the ducts may be more than half the length of the body from the anterior end, as in the young neotenic adult of *Polystoma integerrimum* figured in the present paper (Fig. 7).

The intestinal bifurcation is also relatively distant from the anterior end in *Polystomoides, Neopolystoma, Polystomoidella, Protopolystoma* and *Sphyranura.*

The nephridiopores are set well back in the body among larval forms, becoming more advanced in relative position as the animals age. The excretory pores of *Sphyranura* (Price, 1939, Fig. 1L) and *Protopolystoma* (present paper, Fig. 9) are located at a relatively posterior transverse level, in contrast with those of *Diplorchis* (Ozalci, 1935).

A relatively massive pharynx is typical of larval forms (excepting the newly hatched young of *Sphyranura,* in which development is modified and the gyrodactyloid phase eliminated). The pharynx is comparatively large in the neotenic adult of *Polystoma* (present paper, Fig. 7). A bulky pharynx is also typical of the genera *Polystomoides, Neopolystoma* and *Polystomoidella* (Stunkard, 1916, Price, 1939) and of *Oculotrema* (Stunkard, 1924, Pl. xvi, Fig. 1). A relatively small pharynx characterises *Diplorchis* (Ozaki, 1935), *Polystoma gallieni* (Gallien, 1947) and *P. africanum* (Szidat, 1932).

The body of the adult *Sphyranura* is usually slender. A narrow shape is typical of the larvae of the polystomatid genera, the body becoming relatively wider with increasing length. It is possible that

the slenderness of body in this genus represents a perpetuated larval character.

Corresponding differences in body proportions appear to differentiate the primitive Monogenea from the more advanced members of the group, and the allometric changes during the growth of an individual *Polystoma* possibly reflects a phylogenetic trend.

In the Gyrodactylidae and Dactylogyridae, and in certain other Monopisthocotylea, the pharynx is relatively large and set well back in the body, the eyes (when present), the brain, the nephridiopores and the bifurcation of the gut are relatively posteriorly placed, and generally the proportions of the parts of the body recall those of a young *Polystoma*. It was remarked previously that the adult *Gyrodactylus* bears a strong resemblance to the larvae of polystomes; this similarity includes a correspondence between the proportions of the various parts of the body.

By contrast, in the majority of the Polyopisthocotylea the pharynx is comparatively very small and anteriorly placed, and the intestinal bifurcation, excretory pores, brain, and eyes are relatively well advanced in position.

Thus it seems that polystomatid genera other than those parasitizing terrestrial Anura, like the neotenic adult of *Polystoma*, may possess relatively larval and primitive body proportions. This is perhaps correlated with neotenic evolutionary changes.

The marine Hexabothriidae, grouped together with the Polystomatidae in the superfamily Polystomatoidea, resemble the normal form of *Polystoma*, the main feature of difference being the occurrence in the former of an appendage protruding from the caudal disc. The narrow elongated appendage bears at its free end 1–3 pairs of hooks and a pair of small auxiliary suckers. The Hexabothriidae possess a long, convoluted uterus confined to the preovarian portion of the body, and paired copulation canals. The penis is usually unarmed but is occasionally armed with curved spines, and there is a pair of small cuticularized vaginal openings. The caudal disc bears six suckers, each of which encloses a large hook. The members of this group are parasitic on the gills of elasmobranchs, and are sanguivorous. The proportions of the parts of the body resemble those of large normal individuals of *Polystoma integerrimum*. The life history of the Hexabothriidae is unknown.

The similarity between the marine family and the normal form of the fresh-water genus *Polystoma* suggests a common origin, The normal adult of Polystoma, suggested earlier to represent the least modified form among the Polystomatidae, is also the fresh-water

form which most closely approaches the marine genera in structure, and the characters common to both may offer some indication of the nature of the ancestral stock from which the superfamily Polystomatoidea arose. Knowledge of the life history of the Hexabothriidae would be of interest since this is uncomplicated by the exacting demands of life in a terrestrial host and the need to adapt to the amphibian life cycle.

The ancestral stock evidently possessed a well-developed opisthaptor with six suckers and one or more pairs of hooks, and possibly had both uterus and paired copulation canals. The animals were probably parasitic on the gills of primitive fish ; they were presumably already well adapted to a life of parasitism, and may have fed by sucking blood from the capillaries of the fishes' gills.

The parasites of modern amphibia perhaps remained with the same hosts during the gradual evolution of their terrestrial mode of life. The finely tuned adaptation of the life history of the parasite to the amphibian life cycle was possibly achieved gradually as a result of the parallel evolution of host and parasite.

As the respiratory function in the host was gradually taken over by the lungs, reduction and finally loss of the gills may have caused the parasite to migrate, finally to become established in the bladder. Possibly migration was originally external, by the way of the skin and cloacal opening. The larvae of *Polystoma integerrimum* are in fact able to migrate externally (Williams, 1959a).

As the host became adapted to a terrestrial existence, the conversion during embryonic development from an aquatic to a terrestrial mode of life was presumably progressively accelerated, the aquatic phase being retained as the larval stage of development. So a rapid metamorphosis gradually evolved in the life cycle of the host, involving radical changes in organization, including conversion from a herbivorous to a carnivorous diet with corresponding reorganisation of the alimentary canal. During this transformation the animal undergoes a period of starvation, and the gut is emptied, enabling the internal migration of the parasite to take place.

The trematodes need to lay their eggs in water, the host being infected by the swimming larva. The fact that amphibia lay noncleidoic eggs and must spawn in water was a necessary requirement for the survival of the parasites.

As the amphibian life cycle evolved, it seems that the opportunities for egg laying open to the parasite were restricted to the aquatic larval phase or the limited spawning periods of the adult. By means of the development of a dual life history both of these possibilities

are exploited by the parasite. Thus, dimorphism in *Polystoma* possibly evolved concomitantly with the development of the amphibian life cycle, involving acceleration of development of the form maturing in the tadpole, and retardation of development of the alternative form in the adult frog or toad. The maturation of the bladder form of *Polystoma integerrimum* is delayed in synchronization with the life history of the frog, which does not take part in the Spring migration to the ponds until it is three years old and capable of spawning. Both modes of development appear extreme, and the the neotenic life cycle was probably evolved side by side with the retarded mode of development, in correlation with the amphibian life cycle, rather than interpolated in the "normal" life history.

The larvae embarking on the normal life history are exposed to considerable risk ; many of the larvae are lost from the gill chamber, and there is a heavy mortality among tadpoles and young frogs ; presumably the necessity for migration entails added risk, and in addition many larvae and young worms are lost from the bladder of the young frog during the extended period of development (Zeller, 1872). Thus the probability that any one larvae embarking upon normal development will live to mature and lay eggs is evidently very slight. The neotenic life history avoids most of these risks, and larvae embarking upon neotenic development are much more likely to succeed in laying eggs than are those committed to normal development. Nonetheless, the neotenic adults die before the metamorphosis of the host is completed, and the species must be perpetuated by the larvae undergoing normal development in the bladders of the young frogs.

The branchial form takes approximately one month to reach maturity under laboratory conditions (Zeller, 1876 ; Gallien, 1932a, 1935). The neotenic adult oviposits for approximately thirty days (Gallien, 1935), and may lay as many as thirty-six eggs in twenty four hours (Zeller, 1876). The offspring mainly or exclusively undergo normal development (Gallien, 1934, 1935 ; Savage, 1950). Although the normal adults lay a very large number of eggs, (approximately 1,500 are produced during the Spring season by an animal of medium size ; Zeller, 1876), had the neotenic cycle not evolved considerably fewer larvae would be established in the bladders of the newly metamorphosed frogs. Thus, when a neotenic adult arises from an egg laid by the normal animal in the spawning frog, many larvae embark upon the normal life history whereas otherwise there would have been a single normally-developing larva only. In addition (Savage, 1950), the larvae arising from the eggs of neotenic worms are more likely to establish themselves successfully in the bladder

than are the offspring of normal adults. The former infect tadpoles at a comparatively late stage of development, and so avoid many of the usual hazards, and the worms are less likely to be lost from the branchial chamber or to perish owing to the death of the host.

In brief, adaptation to life in an amphibian host as shown by *Polystoma integerrimum* involves migration at the time of metamorphosis, the evolution of dimorphism, possibly physiological adjustments to immersion in urine, and synchronization of sexual phenomena with the annual cycle of the host.

Whether or not polystomes also infected tailed amphibia cannot be stated ; the only indication that this may have been so is the present association of the aberrant *Sphyranura* with the perennibranch *Necturus*. As the higher vertebrates and modern amphibia evolved, certain groups such as fresh water turtles, the hippopotamus, the Aglossa among the Anura and the Perennibranchiata among the Urodela returned to a greater or less extent to the primitive aquatic environment. The perennibranchiate Urodela became secondarily permanently aquatic as a result of neoteny, remaining in the gilled larval phase of development ; this contrasts with the evolutionary history of the Aglossa, among which the adult form became permanently aquatic.

It seems that turtles and the hippopotamus must have been secondarily parasitized by polystomes which changed hosts at a relatively late stage. The change of host may have involved transfer of the parasites from amphibia, though this can only be conjectured.

The groups suggested earlier to be neotenic may perhaps have evolved through a phase of dimorphic or retarded development in amphibian hosts, in the cases of *Polystomoides* and related genera and *Oculotrema* by a change of host and the perpetuation in the new host of the neotenic form. *Protopolystoma* possibly remained in association with the one host and evolved parallel with it. In the case of *Sphyranura*, although a change of host may have occurred during the evolutionary sequence, there is a further interesting possibility: *Sphyranura* may represent the neotenic form of an originally dimorphic polystome parasitising the tailed amphibian from which *Necturus* evolved. In consequence of the elimination of the adult phase of development of the host by a neotenic evolutionary change, the neotenic form of the parasite alone may survive in the modern perennibranch : i.e., loss of a normal form of the parasite may have been correlated with the elimination of the salamander stage of the host.

The similarly skeletalized caudal suckers of *Oculotrema*, *Sphyranura*, and *Polystomoides* and related genera may reflect a

common origin from a stock distinct from that ancestral to the parasites of tailless amphibia.

It seems from the information available that a lower degree of host specificity is shown by the polystomes parasitic on turtles than by the parasites of modern terrestrial Anura, which are presumably more rigidly adapted to life in their hosts. Thus *Polystomoides coronatum* has been reported from hosts of six different species, *Polystomoidella whartoni* from five different hosts and *Neopolystoma obiculare* from eight species (Price, 1939) ; whereas the polystomes parasitic in amphibia are usually confined to one or two host species. However, the degree of host specificity shown by the polystomatid genera cannot be precisely assessed, since it has proved difficult to establish satisfactory specific criteria within the family. Species have in many cases been described from a single animal or from a small number of specimens ; yet polystomes show considerable individual and seasonal variation, and mature individuals of a species may vary appreciably in size, throughout life, with corresponding differences in the proportions of the various organs and parts of the body. For example, as noted, mature normal individuals of *Polystoma integerrimum* occur from 3 to 13 mm. in length. The anastomosis or pattern of branching of the intestinal crura in *Polystoma integerrimum* is subject to individual variation, and the genital organs, in particular the vitellaria, may undergo extreme seasonal fluctuations. Further, among polystomes parasitic on aquatic reptilia the number of spines in the penial coronet may vary widely (Price, 1939), although this does not apply among the parasites of amphibia.

A further difficulty in evaluating host specificity is that physiological differences may exist between the parasites of different hosts when there is no obvious structural difference.

Throughout the family Polystomatidae, the eggs are normally shed directly or indirectly into the water and sink to the bottom, and generally a fresh host is attacked by the larva. MacCallum (1919), however, records the discovery of turtles, parasitized by *Polystomoides coronatum* and *Neopolystoma orbiculare*, which had diseased lungs containing eggs resembling those of polystomes. A living specimen of *Polystomoides coronatum* was found in the bronchus of one of the turtles. Normally the parasites are confined to the oral and nasal cavities.

It would seem to be an advantage to the worm to parasitize an animal with a permanently aquatic mode of life. Direct infection of the adult stage of the host is then easily accomplished. This eliminates not only the risks involved in migration, but also those due to infection of the vulnerable larval stage of the host ; (however the

hosts may often be more widely dispersed than are many frog and toad tadpoles, and a higher proportion of larvae may fail to find a host). The more or less permanently aquatic hosts harbour parasites at all stages of development ; this contrasts with the condition in the common frog, which when adult is not subject to re-infection by the polystomes. At the time when the larvae of *Polystoma integerrimum* hatch, the frogs have left the pond, and direct infection of the adult does not take place ; (nor are the larvae capable of establishing themselves directly in the frog's bladder when the metamorphosed or adult host is made available to them ; Zeller, 1872). For this reason, and because gradual loss of the parasites from the bladder tends to occur (Zeller, 1872), older frogs are less likely to harbour the polystomes than are younger animals.

However, direct infection of the permanently aquatic Aglossa through the cloacal opening may be possible : a knowledge of the life cycle of *Protopolystoma xenopi* would be of interest from this point of view.

All of the larvae of *Polystoma integerrimum* harboured by a tadpole migrate to the bladder within the space of four or five days at the time of metamorphosis (Gallien, 1932b). The degree of growth and development of the parasite is approximately correlated with the age of the frog, and when two or more polystomes parasitize one host they are typically similar in size and extent of development. Nonetheless, gyrodactyloids and larvae with four suckers may migrate together, and further, as a consequence of competition for food, the more successful individuals may grow and develop more rapidly than their fellows. These factors, possibly combined, may account for the exceptions to the foregoing rule which are occasionally observed. Thus in one extreme case among many groups of two or more worms from one host which were studied, three worms were present in the bladder : one was 5·3 mm. long ; the remaining two were considerably smaller, measuring 2·3 mm. and 1·5 mm. in length, the vaginal prominences of the smallest being only just discernible. These three unequally developed specimens were taken from a sexually mature but small female frog with a body length of 5·9 cm.

As stated earlier, of the three specimens of *Protopolystoma* removed from one host, one, which was sexually mature, was 2·1 mm. long ; the remaining two were immature, both measuring 0·8 mm. in length. The host was a large mature female measuring 7·2 cm. in body length.

It is noteworthy that although the host of the three specimens of *Protopolystoma* was a well-grown individual, two of the parasites were very immature. It seems therefore that the marked difference in size

and degree of development of the three specimens of *Protopolystoma* taken from the same host possibly resulted from factors other than those operating in the case of *Polystoma integerrimum*, and may have been a consequence of direct infection of the adult toad at different times.

Polystoma integerrimum is capable of self-fertilization, but this is apparently not true of certain of the parasites of reptilia (Paul, 1938). The ability to self-fertilize is probably correlated with the fact that infected frogs and toads often harbour a solitary worm.

A further consequence of the permanently aquatic habit of the host is that there is no further need for severe seasonal fluctuations of the genital organs of the parasite. The period of egg laying may be extended, and in correlation with direct infection of the adult host and lowered risk fewer eggs may be laid.

In addition, the polystomes infecting permanently aquatic hosts (including *Protopolystoma*) may mature relatively rapidly, for the development of the parasite need not be delayed to correspond with the time taken by the host to reach sexual maturity.

Neoteny involves the sacrifice of organs formed late in development. Any disadvantages arising from loss or reduction of late-developing structures are balanced against various advantages which vary from group to group, but often include increased evolutionary plasticity and rapid attainment of sexual maturity with early commencement of reproduction. During the evolution of the Polystomatidae, the uterus, absent in Monopisthocotylea, was probably the last of the structures to develop. The vaginae are possibly also relatively recent evolutionary acquisitions. These structures are the last to form during embryonic development, and are therefore the most likely to be affected by a neotenic change. Accordingly, in relation to these characters comparatively recent evolutionary steps may have been retraced.

The presence of a long uterus is to some extent inter-related with the existence of copulation canals. In the absence of vaginae insemination occurs by way of the ovo-vitelline duct ; this process is probably hampered by a long convoluted uterus, and particularly difficult in species lacking a seminal vesicle and without means of storing the introduced sperms for use throughout the time when the uterus is filled with eggs. Thus in forms with a uterus the presence of copulation canals enables insemination to occur continuously throughout the period of egg laying.

Concomitant loss of uterus and copulation canals occurs in the neotenic mode of development of *Polystoma integerrimum*, and may

have occurred during the evolutionary development of *Protopolystoma* ; insemination is correspondingly effected through the shortened ovo-vitelline duct.

As a general rule polystomes which lack uteri parasitize hosts more or less constantly in water ; thus *Protopolystoma* is associated with *Xenopus, Sphyranura* with the perennibranchiate urodele *Necturus, Polystomoides* and related genera with turtles, and the neotenic form of *Polystoma* with the tadpoles of frogs and toads.

A possible explanation for this correlation between the mode of life of the host and the morphology of the parasite is as follows : The existence of a uterus in the normal form of *Polystoma,* and in *Diplorchis* and *Parapolystoma,* is probably correlated with the need to lay a large number of eggs within a limited period. *Polystoma* requires to lay many eggs to compensate for the very high mortality rate among the larvae. The factors contributing towards a high larval mortality rate were listed earlier. Thus a large number of eggs must be laid by the polystomes during the frogs' brief yearly sojourn at the ponds. Eggs may be encapsulated and stored in the uterus of the worm during the migration of the frogs to the pond ; in addition, it may be possible for the eggs to be encapsulated more rapidly if a uterus is present, for the capsules acquire their leather-like consistency while in passage along the uterus, while the following eggs are encapsulated and passed into the uterus in turn. On the other hand among those forms without a uterus each capsule probably tans and hardens while retained within the ootype or oviducal chamber. The egg capsules are typically formed and deposited one at a time, and the rate of egg production is relatively low.

It is possible that a well developed ovicapt is correlated with a long uterus and with the rapid encapsulation of a large number of eggs. A particularly well formed ovicapt occurs in *Parapolystoma bulliense,* while this chamber is lacking in *Protopolystoma xenopi.* The ovicapt is present in the normal form of *Polystoma integerrimum* but is ill-differentiated in the branchial form of this species.

Normal adults of *Polystoma integerrimum* in frogs kept under laboratory conditions lay eggs for an average period of six to eight days ; during this time animals 5-6 mm. in length deposit approximately 1,500 eggs, and double this number may be produced by larger worms (Zeller, 1876). The neotenic adult oviposits for approximately thirty days in the laboratory (Gallien, 1935). Gallien finds that the neotenic adults lay 12-20 eggs per day, while Zeller (1876) states that up to 36 eggs may be deposited in twenty-four hours. Egg production of the normal form is therefore more rapid and of shorter duration than that of the neotenic form.

In *Polystomoides oris* egg production occurs throughout the year; an individual of this species lays 2-3 eggs in twenty-four hours (Paul, 1938).

In contrast with the forms parasitic in the urinary bladders of terrestrial Anura, the worms with permanently aquatic hosts probably lay relatively few eggs over an extended period. In correlation with this the uterus may have been sacrificed.

Hence, accelerated development and rapid attainment of sexual maturity, reduction of the vaginae, loss of the uterus, and the permanently aquatic habit of the host may be interdependent features.

Summary

A redescription is given of the morphology of the bladder (normal) and branchial (neotenic) forms and the structure of the larva of *Polystoma integerrimum*, including a description of the histological changes accompanying digestion, and an account of the peripheral sensory system of the bladder adult.

In both adult forms, and in larvae established in the branchial chamber, the pharynx is underlain by a continuous canal (named the bucco-intestinal canal) formed by the junction of a median anterior intestinal process with the buccal tube.

In the bladder adult, the ootype leads into a large chamber (for which the term oviducal chamber is suggested), the walls of which contain a series of well-defined sphincter muscles. The oviducal chamber inclines towards the ventral body wall and opens into the long winding uterus. Cross insemination, or auto-insemination in solitary animals, occurs through the paired vaginae.

Associated with the ootype are two distinct gland masses, one of which is evidently mucus-secreting; both types of gland are present also in the branchial form.

The branchial adult is neotenic, being a product of accelerated and incomplete but otherwise little modified normal development together with the relatively early attainment of sexual maturity. The most important manifestation of neotenic development in *Polystoma integerrimum* is the incomplete formation of the female genital duct system in the branchial form, both uterus and vaginae, which arise late in the normal ontogeny, failing to develop. The oviducal chamber opens directly into the genital atrium. The vas deferens and the genito-intestinal canal are present and functional. There is no

internal communication between the male and female organs, and insemination must take place by way of the short ovo-vitelline duct. The primitive mode of insemination is therefore readopted by the branchial form of this species, as a consequence of structural simplification accompanying neotenic development.

An account is given of the morphology of a polystome parasitizing *Xenopus laevis* Daud. The species is provisionally identified with *Protopolystoma xenopi* (Price) Bychovsky pending further study of this species. *Protopolystoma* is characterized by the absence of uterus and vaginae and the possession of caudal suckers which are not heavily skeletalized or divided into annular zones, and inhabits the urinary bladder of Aglossa. In the species studied a bucco-intestinal canal is present, and both gland masses associated with the ootype in *Polystoma integerrimum* are represented. A capacious oviducal chamber opens directly into the genital atrium. As in the neotenic form of *Polystoma*, insemination must occur through the ovo-vitelline duct.

Protopolystoma, having fundamentally the same structure as the neotenic adult but the location within the host of the normal form of *Polystoma*, may be neotenic. All other genera parasitic in the bladder of amphibia possess the full complement of genital ducts.

Neotenic changes may have occurred independently along several lines of evolution in the phylogeny of the Polystomatidae. The absence of a true uterus in *Polystomoides*, *Neopolystoma* and *Polystomoidella*, and perhaps also the absence of vaginae in *Oculotrema*, may be secondary simplifications of the genital duct system resulting from neoteny.

Sphyranura lacks a uterus and may also have reduced vaginae. In addition, *Sphyranura* has a single pair of caudal suckers, larval hooklets which are retained throughout life, a slender larval shape and a short life span; these characters may be correlated with neotenic development.

The proportions of the various organs and parts of the body in the neotenic form of *Polystoma integerrimum* are more similar to the proportions characteristic of the larvae than are those of the normal adult. Similarly, proportions characteristic of an early stage of development (and possibly also of a primitive evolutionary phase) distinguish those genera suggested above to be neotenic.

The normal forms of *Polystoma* and the Hexabothriidae, a marine family parasitic on the gills of elasmobranchs, show a similarity in structure which possibly indicates that these forms stemmed from a common ancestral stock parasitic on the gills of primitive fish. The

polystomatid genera parasitic in Anura may have evolved parallel with their hosts as the latter became adapted to a terrestrial existence. Dimorphism, with retarded and accelerated modes of development ; migration to the bladder at the time of metamorphosis ; and seasonal ripening and collapse of the genital organs, are adaptations to the life history of the host acquired by the parasite.

Polystomes may also have infected the tailed amphibia : *Sphyranura*, parasitic on a perennibranchiate urodele, may represent the neotenic form of an originally dimorphic polystome with a normal form harboured by the salamander stage of the host.

Polystomoides and related genera parasitize aquatic reptiles, while *Oculotrema* lives on the eye of the hippopotamus. Polystomes infecting aquatic hosts have the advantage of being exposed to relatively little risk throughout their life histories ; their life cycles may be simple, and development need not be retarded.

A permanently neotenic condition in the parasite appears to be correlated with an aquatic mode of life of the host. The presence of a uterus in species inhabiting the bladders of terrestrial Anura is probably related to the need to lay numerous eggs during a limited period of time. The absence of a uterus is not an appreciable disadvantage to polystomes infecting hosts constantly immersed in water, for the parasites are able to oviposit over an extended period. In the absence of a uterus insemination through the ovo-vitelline duct is easily accomplished and reduction of the vaginae would not be detrimental. Thus among polystomes parasitic on permanently aquatic hosts there may have been a tendency for these late-developing organs to be sacrificed, which in correlation with the opportunity for rapid maturation may have led to neotenic development.

Acknowledgments

This work was carried out at the London School of Hygiene and Tropical Medicine (Keppel Street, London W.C.1), under the supervision of Professor J. J. C. Buckley, to whom the author is indebted for helpful suggestions and criticisms.

References

Alvey, C. H., 1933.—"The life cycle of *Sphyranura oligorchis*." *J. Parasit.*, **20,** 140. (W.L. 11428).

————, 1936.—"Morphology and development of the monogenetic trematode *Sphyranura oligorchis*, with a description of *S. polyorchis* sp. nov." *Parasitology*, **28,** 229–253. (W.L. 16035).

André, J., 1910 (a).—"Zur Morphologie des Nervensystems von *Polystomum integerrimum* Fröhl." *Z. wiss. Zool.*, **95,** 191–202. (W.L. 23635).

————, 1910 (b).—"Die Augen von *Polystomum integerrimum* Fröhl." *Z. wiss. Zool.*, **95,** 203–220. (W.L. 23635).

Bychovsky, B. E., 1957.—"The monogenetic trematodes, their systematics and phylogeny." *Zool. Inst. Acad. Sci. Moscow-Leningrad* 1957, pp. 509. (Russian).

Cernosvitov, L., 1931.—"Studien über die Spermaresorption. III. Die Samenresorption bei den Tricladen." *Zool. Jb.*, (2) **54,** 295. (W.L. 23831).

De Beer, G. R., 1940.—Embryos and ancestors. Oxford.

Deegan, T. and Maegraith, B. G., 1956 (a).—"Studies on the nature of the malarial pigment (haemozoin). 1. The pigment of the simian species, *Plasmodium knowlesi* and *P. cynomolgi*." *Ann. trop. Med. Parasit.*, **50,** 194. (W.L. 1063).

————, 1956 (b).—"Studies on the nature of the malarial pigment of the human species, *Plasmodium falciparum* and *P. malariae*." *Ibid.*, **50,** 212.

Faust, E. C., 1918.—"Eye-Spots in Digenea." *Biol. Bull., Woods Hole*, **35,** 117–127. (W.L. 2975).

Gallien, L., 1932 (a).—"Sur la reproduction néoténique chez *Polystomum integerrimum* Fröhlich." *C. R. Acad. Sci., Paris*, **194,** 1852–1854. (W.L. 6628).

————, 1932 (b).—"Sur l'évolution de la génération issue des formes néoténiques de *Polystomum integerrimum* Fröhlich." *Ibid.*, **195,** 77–79.

————, 1933.—"Transformations histologiques correlatives du cycle sexual chez *Polystomum integerrimum* Fröhlich." *Ibid.*, **196,** 426–428.

————, 1934.—"Sur le déterminisme de la dualité d'évolution des larves chez *Polystomum integerrimum* Fröhlich." *Ibid.*, **199,** 229–231.

————, 1935.—"Recherches expérimentales sur le dimorphisme évolutif et le biologie de *Polystomum integerrimum* Fröhl." *Trav. Sta. zool. Wimereaux* **12,** 181 pp. (W.L. 21765 f).

————, 1938.—"Sur un polystome parasite de *Hyla arborea* L. var. *meridionalis* Boettgr." *Bull. Soc. zool. Fr.*, **63,** 251–254. (W.L.5401).

————, 1947.—"Description de *Polystoma gallieni* E. W. Price parasite de *Hyla arborea* var. *meridionalis* Boettgr." *Ann. Parasit. hum. comp.*, **22,** 24–29. (W.L. 899 a).

Goto, S., 1891.—"On the connecting canal between the oviduct and the intestine in some monogenetic trematodes. (Preliminary communication)." *Zool. Anz.*, **14,** 103–104. (W.L. 23833).

Grobbelaar, C. S., 1922.—"On South African Paramphistomidae. II. Some trematodes in South African Anura, and the relationships and distribution of their hosts." *Trans. roy. soc. S. Afr.*, **10,** 181–200. (W.L. 21653).

Halkin, H., 1902.—"Recherches sur la maturation, la fécondation et la développement du *Polystomum integerrimum*." *Arch. Biol., Paris*, **18,** 291–363. (W.L. 1825).

HESSE, R., 1897.—"Untersuchungen über die Organe der Lichtemfindung bei niederen Thieren. II. Die Augen der Plathelminthen, insonderheit der tricladen Turbellarien." *Z. wiss. Zool.*, **62,** 527–582. (W.L. 23635).

HYMAN, L. H., 1951.—The invertebrates : Platyhelminthes and Rhynchocoela. **2.** New York, Toronto and London.

IJIMA, I., 1884.—"Über den Zusammenhang des Eileiters mit dem Verdauungscanal bei gewissen Polystomeen." *Zool. Anz.*, **7,** 635–639. (W.L. 23833).

JENNINGS, J. B., 1959.—"Studies on Digestion in the Monogenetic Trematode *Polystoma integerrimum*." *J. Helminth.*, **33,** 197–204. (W.L. 11224c).

JOHNSTON, S. J., 1912.—"On some trematode parasites of Australian frogs." *Proc. Linn. Soc. N.S.W.*, **37,** 285–362. (W.L. 16790a).

LEMBERG, R. and LEGGE, J. W., 1949.—Haematin compounds and bile pigments : their constitution, metabolism and function. New York and London.

LLEWELLYN, J., 1954.—"Observations on the food and gut pigment of the Polyopisthocotylea (Trematoda : Monogenea)." *Parasitology*, **44,** 428–437. (W.L. 16035).

LOPEZ-NEYRA, C. R., 1952.—"*Polystoma palancai* n.sp. (Monogenea : Polystomatidae), parasita de la vejiga urinaria de la rana de San Antón." *Rev. ibér. Parasit.*, **12,** 289–295. (W.L. 18858c).

MACCALLUM, G. A., 1919.—"Studies on the Polystomidae." *Zoopathologica*, **1,** 101–120. (W.L. 23839).

ODHNER, T., 1912.—"Die Homologien der weiblichen Genitalwege bei den Trematoden und Cestoden. Nebst Bemerkungen zum natürlichen System der monogenen Trematoden." *Zool. Anz.*, **39,** 337–351. (W.L. 23833).

————, 1913.—"Noch einmal die Homolgien der weiblichen Genitalwege der monogenen Trematoden." *Ibid.*, **41,** 558–559.

PAGENSTECKER, H. A., 1857.—Trematodenlarven und Trematoden, helminthologischer Beitrag. Heidelberg.

PAUL, A. A., 1938.—"Life history studies of North American fresh-water polystomes." *J. Parasit.*, **24,** 489–510. (W.L. 11428).

PRENANT, M., 1922.—"Recherches sur le parenchyme des Platyhelminthes." *Arch. Morph. gén. exp.*, **5,** 175 pp. (W.L.1873).

PRICE, E. W., 1939.—"North American monogenetic trematodes. IV. The family Polystomatidae (Polystomatoidea)." *Proc. helm. Soc. Wash.*, **6,** 80-92. (W.L. 16747a).

————, 1943.—"A new trematode of the genus *Polystoma* (Monogenea : Polystomatidae) from *Xenopus laevis* Daud." *Ibid.*, **10,** 83–85.

SAVAGE, R. M., 1950.—"Observations on some natural epizootics of the trematode *Polystoma integerrimum* among tadpoles of *Rana temporaria temporaria*." *Proc. zool. Soc. Lond.*, **120,** 15–37. W.L. 16969b).

STEEDMAN, H. F., 1950.—"Alcian blue 8 GS : a new stain for mucin." *Quart. J. micr. Sci.*, **91,** 477–480. (W.L. 17510).

STEPHENSON, W., 1947.—"Physiological and histochemical observations on the adult liver fluke *Fasciola hepatica* L. III. Egg shell formation." *Parasitology*, **38,** 128–139. (W.L. 16035).

STEWART, F. H., 1914.—"The anatomy of *Polystomum kachugae* sp.nov. with notes on the genus *Polystomum*." *Rec. Indian Mus.*, **10,** 195–205. (W.L. 17746).

STUNKARD, H. W., 1917.—"North American Polystomidae, Aspidogastridae, and Paramphistomidae." *Illinois biol. Monogr.*, **3,** 283–394. (W.L. 9222).

————, 1924.—"A new trematode, *Oculotrema hippopotami*, n.g., n.sp., from the eye of the hippopotamus." *Parasitology*, **16,** 436–440. (W.L. 16035).

SZIDAT, L., 1932.—"Parasiten aus Liberia und Französisch-Guinea. II. Trematoden." *Z. Parasitenk.*, **4,** 506–521. (W.L. 23536b).

WILLEMOES-SUHM, R., 1872.—"Zur Naturgeschichte des *Polystomum integerrimum* und des *P. ocellatum* Rud." *Z. wiss. Zool.*, **22,** 29–39. (W.L. 23635).

WILLIAMS, J. B., 1957.—"Anatomy of *Polystoma integerrimum*." *Nature, Lond.*, **180,** 866. (W.L. 14900).

————, 1959a.—"Abnormal Migration in *Polystoma integerrimum*." *J. Helminth.*, **33,** 205-206. (W.L. 11224c).

————, 1959b.—"Preliminary Note on the Anatomy of a Polystome from the Bladder of *Xenopus laevis* Daud. *Ibid.*, **33,** 207–208.

WRIGHT, R. R. and MACCALLUM, G. B., 1887.—"*Sphyranura osleri* a contribution to American helminthology." *J. Morph.*, **1,** 1–48. (W.L. 11373).

ZELLER, E., 1872.—"Untersuchungen über die Entwicklung und Bau des *Polystomum integerrimum* Rud." *Z. wiss. Zool.*, **22,** 1–24. (W.L. 23635).

————, 1876.—"Weiterer Beitrag zur Kenntniss der Polystomen." *Ibid.*, **27,** 238–274.

Journal of Helminthology, Vol. XXXV, Nos. 1/2, 1961, *pp.* 203–206.

Oesophagostomum watanabei n.sp. (Nematoda, Strongylidae) from a Japanese Wild Boar

By Satyu Yamaguti

Zoological Institute, Faculty of Science, Kyoto University, Kyoto, Japan

Seven mature males and as many females, mature or gravid, were taken from the large intestine of *Sus leucomystax* Temm. by Dr. Shozo Watanabe of the Institute of Veterinary Hygiene in Niigata Prefecture on 2nd March, 1940. A careful comparison of them with related species has revealed that they represent a distinct species, for which the name *Oesophagostumum watanabei* is proposed in honor of the collector.

Male. Body 7·0–8·5 mm. long by 0·43–0·47 mm. wide. Mouth collar 0·16 mm. in diameter at base. Two pairs of submedian head papillae projecting only slightly over the cuticle, but not so prominently as in *O. dentatum*. External leaf-crown with 28–31 narrow leaflets, internal with 56–62 minute dentiform elements. The cuticle in front of the ventral cervical groove is inflated and extremely finely striated transversely and does not form a distinct vesicle. The cervical groove is well developed ventrally and extends round the sides to the dorsal surface where it appears as a shallow constriction ; it lies 0·15–0·18 mm. from the head end distinctly anterior to the nerve ring, the latter being 0·22–0·25 mm. behind the head end. Cervical papillae are a little anterior to middle of posterior oesophageal swelling, about 0·4 mm. from head end. The cephalic glands extend backward beyond the cervical groove but not so far as they do in *O. dentatum* (cf. Goodey, 1924, Fig. 4). The lateral cervical glands, each containing very fine, densely packed granules and a large nucleus, are elongated club-shaped and extend from the excretory pore to the anterior testicular coils, the shorter left gland being 1·8–1·95 mm. long and the right 2·3–2·6 mm. long.

Buccal capsule ring-shaped, 0·1–0·12 mm. in outside diameter. Oesophagus vase-shaped 0·53–0·57 mm. long, constricted in region of nerve ring; anterior swelling 0·14–0·16 mm. wide; posterior swelling elliptical, 0·17–0·2 mm. wide, followed by a tri-valve. Oesophageal lumen triradiate, each chamber being lined with very thick cuticle, which appears moniliform in optical section in the constricted region. Anterior part of intestine showing conspicuous intercellular constriction. Testis originating at level of seminal vesicle, 0·32–0·34 mm. from head end, winding around intestine as far forward as posterior end of cervical glands, where it turns backward and becomes gradually wider to be continued into an elliptical seminal vesicle; the latter is 0·35–0·45 mm. long by 0·15–0·2 mm. wide and studded with rounded, finely granular spermatozoa. Prebursal papillae slender; bursa comparatively short, consisting of broadly rounded or truncate dorsal lobe and semicircular lateral lobes, with a distinct notch between dorsal and lateral lobes; ventral ray cleft to base; three lateral rays with common trunk, externolateral divergent from mediolateral, terminating on external surface of bursa about 35μ from its margin; mediolateral and posterolateral close together, reaching to margin; posterolateral with an accessory twig arising from its base and directed posterodorsally; externodorsal arising from common trunk with dorsal, and terminating submarginally in lateral lobe; dorsal bifurcated about its middle, each branch reaching to margin of dorsal lobe, with a ventral twig near its base and another smaller ventromedial twig a little more distally. Spicules equal, narrow, alate, 0·83–0·9 mm. long, 40–50μ wide. Gubernaculum about 0·15 mm. long, consisting of a heavily sclerotized scoop-shaped anterior portion without handle, and a poorly sclerotized posterior portion, the shape of which I was unable to determine.

Female. Body 6.85–8·0 mm. long by 0·38–0·5 mm. wide. Mouth collar 0·16–0·18 mm. in diameter; buccal capsule 0·11–0·12 mm. in outside diameter. Excretory pore, nerve ring and cervical papillae 0·14–0·18 mm., 0·23–0·28 mm. and about 0·4 mm. respectively from head end. Left cervical gland 1·9–2·1 mm. long, reaching anterior end of ovary, right one 2·4–2·9 mm. long. Oesophagus 0·53–0·6 mm. long, 0·14–0·16 mm. wide at anterior swelling, 0·17–0·2 mm. wide at posterior swelling. Paired ovijectors about 50μ wide, vagina 0·12 mm. long; vulva protuberant in some specimens, 0·27–0·35 mm. anterior to anus. Tail 0·23–0·27 mm. long, tapering abruptly a little behind anus, directed straight backward and ending in a slender tip. Eggs elliptical, measuring *in situ* 75–88μ long by 32–38μ wide, each containing a segmenting ovum.

This species is characterised by the posterolateral bursal ray being provided with an accessory twig near its base. That the cervical groove lies distinctly anterior to the nerve ring in the present species is another important character differentiating it from the related species parasitic in *Sus*.

The principal measurements (in mm.) of the present species are compared with those of the related species in the following table.

	O. dentatum [1] (Rud., 1803)	*O. brevicaudum* Schw. et Alic. 1930	*O. longicaudum*[2] Goodey, 1925	*O. watanabei* n.sp.
Body length ♂	8–12	6·2–6·8	8–10	7·0–8·5
♀	8–15	6·4–8·5	11–14	6·85–8·0
Number of external leaf-crown elements	9–10	14–16	9(?10)	28–31
Tail of female	0·23–0·53	0·081–0·12	0·4–0·49	0·23–0·27
Vulva from anus	0·3–0·49	ca. 0·1	0·38–0·46	0·27–0·35
Spicules	0·88–1·3	1·05–1·23	0·8–0·95	0·83–0·9
Eggs	61–74× 36–42μ	52·5–67·4 × 30–45μ	?	75–88× 32–38μ

[1] Measurements combined from those by Goodey (1924), Yorke and Maplestone (1926), and Yamaguti (1935).

[2] Measurements combined from those of Marcone (1901) for *O. quadrispinulatum* and Goodey (1925); cf. Alicata (1935).

REFERENCES

ALICATA, J. E., 1935.—"*Oesophagostomum longicaudum* Goodey, 1925, a synonym of *Oesophagostomum quadrispinulatum* (Marcone, 1901)." *J. Parasit.*, **21**, 215–216. (W.L. 11428).

GOODEY, T., 1924.—"The anatomy of *Oesophagostomum dentatum* (Rud.), a nematode parasite of the pig, with observations on the structure and biology of the free-living larvae." *J. Helminth.*, **2**, 1–14. (W.L. 11224c).

————, 1925.—"*Oesophagostomum longicaudum* n.sp. from the pig in New Guinea." *J. Helminth.*, **3**, 45–50. (W.L. 11224c).

Schwartz, B. and Alicata, J. E., 1930.—"Two new species of nodular worms (*Oesophagostomum*) parasitic in the intestine of domestic swine." *J. agric. Res.*, **40,** 517–522. (W.L. 10966)

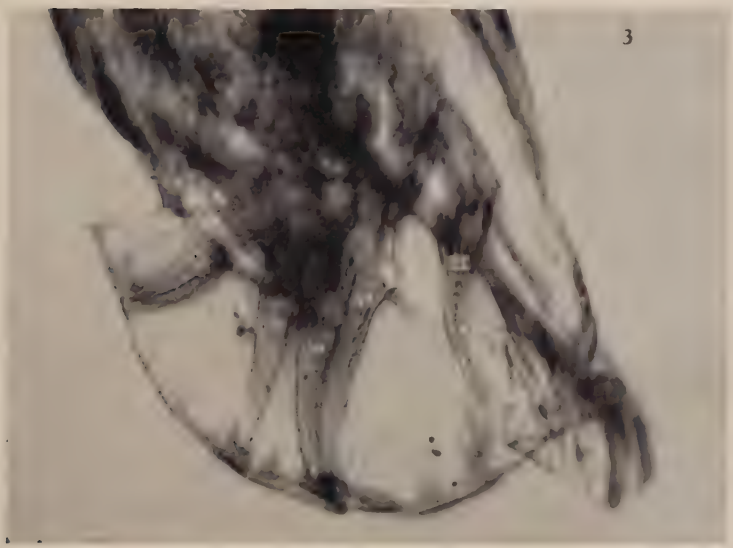
Yamaguti, S., 1935.—"Studies on the helminth fauna of Japan. Part 13. Mammalian nematodes." *Jap. J. Zool.*, **6,** 433–457. (W.L. 10881w)

Oesophagostomum watanabei n.sp.

Fig. 1.—Anterior extremity, lateral view. **CG**=cervical groove. **N**=nerve ring. Fig. 2.—Posterior extremity of female, lateral view. **A**=anus, **I**=intestine, **OJ**=ovijector, **V**=vulva. Fig. 3.—Posterior extremity of male, lateral view. **ED**=externodorsal ray, **EL**=externolateral ray, **ML**=mediolateral ray, **PL**=posterolateral ray with accessory twig, **V**=ventral rays.

1
CG

2
A


3

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 207–208.

The First Record of Human Infection with *Bertiella studeri* in Malaya

By R. S. Desowitz, H. J. Wong and M. A. Fernando

Department of Parasitology, University of Malaya, Singapore, and Paediatrics Unit, General Hospital, Singapore

Although *Bertiella studeri* is a rare parasite of man it appears to have a widespread distribution throughout the tropics. Four cases have been reported from Mauritius (Blanchard, 1913 ; Adams and Webb, 1933 ; Adams, 1935), four cases from India (Chandler, 1925 ; Mukerji, 1927 ; Maplestone, 1930 ; Maplestone and Riddle, 1936), one from Sumatra (Joyeux and Dollfus, 1931), three cases from Indonesia (Bonne, 1940 ; Lie Kian Joe, 1960, personal communication), one in Borneo (Lie Kian Joe, 1960, personal communication), one from the Phillipines (Africa and Garcia, 1935), one from Kenya (Buckley and Fairley, 1950) and one from St. Kitts, B.W.I. (Cameron, 1929). A related species, *B. mucronata* was reported as infecting a man in Cuba (Cram, 1928) and a man in Paraguay (Bacigalupo, 1949). These two cases are of interest in that adults were infected whereas all the cases of *B. studeri* have been children. The present case, also an infection in a child represents the first record of this parasite occurring in Malaya.

Case History : A six year old Chinese child living in Singapore was admitted to the children's ward of the General Hospital after his mother noticed tapeworm segments in his stool. The patient had no complaints of abdominal pain but was anorexic and lost weight a week or two before admission to hospital. Stools were normal in consistency. No other members of the family were found to be infected with tapeworm. The child was found to be infected with hookworm and *Ascaris*.

Segments of 10-12 gravid proglottids were found in the stool. A rectal saline washout was administered and another portion of the worm obtained. The mature and gravid proglottids as well as eggs obtained from the latter were typical of *Bertiella studeri*. A dose of 1.5 gms. Dichlorophen (5 : 5 dichloro—2 : 2 dihydroxydiphenylmethane) was given orally and a degenerated tapeworm measuring 10 cm. was passed. The scolex was not found. No recurrence of infection has been noted three months after treatment.

The faeces of the family's pet dog was examined since these animals can be infected with the parasite and together with monkeys might constitute the normal hosts (Africa and Garcia, 1935). The faeces of this animal were negative on all occasions when examined.

Summary

1. A six year old child living in Singapore was found to be infected with the cestode *Bertiella studeri*.

2. Apparent cure was affected by use of Dichlorophen.

References

Adams, A. R. D., 1935.—"A fourth case of human infestation with *Bertiella studeri* in Mauritius". *Ann. trop. Med. Parasit.*, **29,** 361–362. (W.L. 1063).

———— and Webb, L., **1933.**—"Two further cases of human infestation with *Bertiella studeri* (Blanchard 1891) Stiles and Hassall, 1902, with some observations on the probable synonymy of the specimens previously recorded from man." *Ibid.*, **27,** 3.

Africa, C. and Garcia, E. Y., 1935.—"The occurrence of *Bertiella studeri* in man, monkey and dog in the Phillipines". *Philip. J. Sci.*, **56,** 1–12. (W.L. 16189).

Bacigalupo, J., 1949.—"Primer caso humano de *Bertiella* sp. en Sud America" *Rev. Soc. mex. Hist. nat.*, **10,** 177–183. (W.L. 18951d).

Blanchard, R., 1913.—"*Bertiella satyri*, de l'orang-outang et aussi parasite de l'homme". *Bull. Acad. Méd., Paris*, **69,** 286. (W.L. 3683).

Bonne, C., 1940.—"Over *Bertiella studeri* (Blanchard 1891). *Bertiella satyri* Stiles and Hassall 1926. *Bertiella satyri* Blanchard 1891." *Geneesk. Tijdschr. Ned.-Ind.*, **80,** 2222–2230. (W.L. 9002).

Buckley, J. J. C. and Fairley, N. H., 1950.—"Demonstration of a Cestode, *Bertiella*; a rare parasite of man and recorded for the first time as a human infection in Africa." *Trans. R. Soc. trop. Med. Hyg.*, **44,** 2. (W.L. 21671).

Cameron, T. W. M., 1928.—"A new record of the occurrence of tapeworm of the genus *Bertiella* in man." *J. Helminth.*, **7,** 231–234. (W.L. 11224c).

Chandler, A. C., 1925.—"New records of *Bertiella satyri* (Cestoda) in man and apes." *Parasitology*, **17,** 421–425. (W.L. 16035).

Cram, E. B., 1928.—"A species of the Cestode genus *Bertiella* in man and the chimpanzee in Cuba." *Amer. J. trop. Med.*, **8,** 339–344. (W.L. 626).

Joyeux, Ch. and Dollfus, R. B., 1931.—"Un nouveau cas de *Bertiella studeri* chez l'homme." *C.R. Soc. Biol. Paris.*, **107,** 35–36. (W.L. 6630).

Maplestone, P. A., 1930.—"A new case of *Bertiella* in a human being." *Indian Med. Gaz.*, **65,** 258–260. (W.L. 9943).

———— and Riddle, J. S., 1936.—"Infection with *Bertiella studeri*." *Ibid.*, **71,** 81.

Mukerji, A. K., 1927.—"The incidence of helminthic infections in the Carmichael Hospital for Tropical Diseases, Calcutta." *Ibid.*, **62,** 695–696.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 209–220.

On *Brugia (Brugiella* subgen. nov.*) buckleyi* n.sp., from the Heart and Blood Vessels of the Ceylon Hare

By A. S. Dissanaike and D. C. Paramananthan*

Department of Parasitology, Faculty of Medicine, University of Ceylon, Colombo

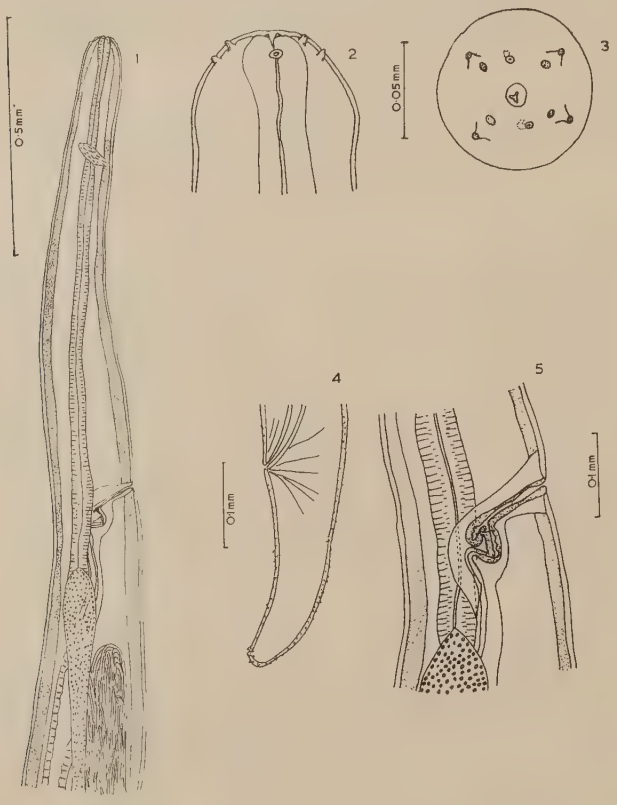
As reported by us in a previous communication (Dissanaike and Paramananthan, 1961) *Brugia* type microfilariae and the corresponding adult worms were discovered from a Ceylon Hare (*Lepus nigricollis singhala*). This hare was shot at Mundumurippu in the Northern Province of the island and the adult worms were found in the heart and hepatic blood vessels. Three complete mature females and three complete male specimens as well as several damaged specimens of both sexes formed the collection from this hare. They were found to differ distinctly from the hitherto described species of *Brugia*. It is therefore proposed to place it in a new subgenus, *Brugiella* and to name it *Brugia* (*Brugiella* subgen. nov.) *buckleyi* n.sp. in honour of Professor J. J. C. Buckley who has been responsible for the separation of the genus and to whom we are grateful for the invaluable help and suggestions he has given.

We have now collected several more specimens of this helminth from the Northern, North Central and North Western Provinces of the island and these have also been from the heart and hepatic blood vessels, although one specimen was recovered from the posterior vena cava. On a previous occasion we had recovered the adults of another filarioid from two different hares (Hares No. 1 and 2). We have identified it as *Micipsella* sp. and shall deal with it in a later paper. The blood of these hares contained sheathed microfilariae with a characteristic "tail-cap" nucleus. On re-examination of this material we found that in Hare No. 1, three immature female specimens of *Brugia* were present in addition to the adult *Micipsella*.

From 10 hares examined so far, we have collected complete and incomplete specimens of 15 female and 8 male worms. Four of these

* Ceylon Association for the Advancement of Science, Research Student.

hares were positive for microfilariae of the *Brugia* type. In one of these microfilaria-positive hares only immature female worms were recovered (Hare No. 7) while in another, only incomplete male



Brugia (*Brugiella*) *buckleyi* n.sp.

Fig. 1.—Anterior extremity of a female, lateral view. Fig. 2.—Lateral view of head end. Fig. 3.—End-on view of head. Fig. 4.—Lateral view of female tail. Fig. 5.—Vulva, ovejector and part of vagina.

specimens were found (Hare No. 9). This seems to suggest that we have missed mature female specimens from these latter animals, and it is possible that the adults are to be found in some other sites as well. In future, therefore, it will be necessary to search for the

adult worms in the lymphatics and lymphatic glands in addition to the heart and hepatic blood vessels.*

Brugia (*Brugiella*) *buckleyi* n.sp.
Figs. 6–9.—Tails of 4 different male specimens, left lateral view.

Hare No. 3 which had large numbers of microfilariae of the *Brugia* type in the blood, had a few microfilariae of the *Micipsella* sp. as well. Although complete adult worms of the *Micipsella* sp. were not recovered from this hare we found what appeared to be dead and degenerate specimens from a blood clot in the hepatic blood vessels.

* Since this paper went to press over 30 hares examined from different parts of Ceylon have been found to be positive. The right ventricle and the pulmonary arteries were found to be the commonest sites for the adult worms.

Description

Tables 1–3 below summarize the main dimensions of the complete and incomplete, mature and immature worms so far collected.

The Female.

The principal measurements of mature specimens are as follows : Length, 82–94 mm.; breadth, (maximum), 300–350μ ; oesophagus, 950–1170μ long ; vulva (from anterior end), 820–1140μ ; tail, 180–250μ long ; diameter of "head bulb", 62–88μ.

Cuticular bosses are quite distinctly seen in the posterior region of the female. Of the phasmids described by Buckley *et. al.* (1958), only a left sub-dorsal one can be recognised in one specimen.

The Male.

Length, 41–50 mm.; breadth, 170–210μ ; left spicule, 267–285μ long ; right spicule, 127–150μ long ; spicule ratio, 1·8—2·1 : 1 ; tail, 185–230μ long ; "head bulb", 55–80μ.

The caudal papillae in the male. A large unpaired pre-anal papilla is present. The adanal papillae differ in number and general arrangement from those of the Malayan and African species. There are generally 3 to 6 pairs of lateral papillae including the post-anals, which are not close together as in the other species.

There are one or two pairs of lateral papillae lying between the adanal group and the tip of the tail. They are generally asymmetrical and their position is variable. These intermediate papillae are quite close to the adanal group in some specimens (Fig. 14).

The pair of papillae at the tip of the tail may lie more anteriorly.

The Microfilariae.

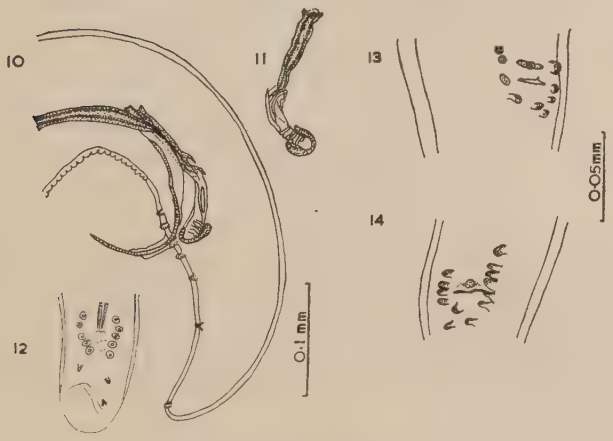
The sheathed microfilariae are of the typical *Brugia* type, most of them lying in irregular curves, with coarse overlapping nuclear columns and showing the usual landmarks. They measure between 135–225μ (average 174·5μ) in length by 4·5–6·3μ (average 5·6μ in breadth). The head space varies from 3–6μ (average 4·7μ) in length. The terminal and sub-terminal tail nuclei are often rounded and contained in a bulbar swelling, but one or both of these nuclei may be elongate (Plate I, Fig. B).

Host : Ceylon Black-naped Hare (*Lepus nigricollis singhala*).

Habitat : Heart and blood vessels.

Locality : Northern parts of Ceylon.

Types : In the Department of Parasitology, Faculty of Medicine, University of Ceylon, Colombo.



Brugia (*Brugiella*) *buckleyi* n.sp.

Fig. 10.—Tail of male with everted spicule, left lateral view. Fig. 11.—Right spicule from right side. Fig. 12.—Ventral view of tail end of male (not to scale). Fig. 13 and 14.—Sub-ventral views of 2 male specimens showing variation in number and arrangement of adanal papillae.

Brugiella subgen. nov.

Relatively large worms with the characters of the genus *Brugia* but without distinct head bulb ; with shorter oesophagus and with vulva close to posterior extremity of oesophagus ; with smaller spicule ratio ; with male caudal papillae different in number and arrangement ; parasitic in heart and blood vessels.

Discussion and Relationships

It is clear from the above description that *B. buckleyi* differs from the three species described so far chiefly in size. In *B. malayi*, *B. pahangi* and *B. patei*, the females are all below 60 mm. in length and 190μ in maximum breadth and the males are below 25 mm. in length and 100μ in maximum breadth (Buckley, 1960). The males and females of *B. buckleyi* n.sp. are almost twice as long and are much broader. In this respect this species comes closer to *W. bancrofti*. Together with this greater size of the worms go dimensions of other structures such as the "head bulb" and the relative lengths of the tails of both sexes. An interesting point in *B. buckleyi* n.sp. is that there is no definite "head bulb" as such, and the measurements in the tables merely show the maximum diameter of the head end. Furthermore the "bulb" at the head end is seen below the level of the 4 sub-lateral papillae (Fig. 2) unlike the other three species where it is in the region of the sub-lateral papillae. It is also noticed that in the end-on view the "head bulb" is circular and not oblong so that the diameter is the same dorsoventrally as well as laterally.

One other striking feature is that the oesophagus, particularly in the male, appears to be much shorter than in all the other species although the worms themselves are several times larger. In the female, the relative closeness of the vulva to the end of the oesophagus distinguishes this species from all the other known species. In fact in one specimen the vulva is behind the end of the oesophagus.

All other species of *Brugia* differ significantly from *B. buckleyi* in the spicule ratio, for in *B. buckleyi* the ratio is on the average 2 or less than 2 : 1, while in the others it is more than 2·2 : 1. The wide separation of the postanal papillae further distinguishes this species.

B. buckleyi n.sp. resembles *B. pahangi* and *B. patei* in the fact that the left spicule is about the same order of size ; but whereas the male of *B. malayi* is smaller in size the left spicule is very much longer.

B. buckleyi differs from *B. patei* in the possession of prominent cuticular bosses at the posterior end of the female and also in the absence of a cup shaped base and a spatulate tip to the left spicule. In this respect it simulates the other two species.

Table I

Females (Immature)

Hare No.	1	1	1	5	5	5	6	7	7
Specimen No.	1	2	3	9	10	11	12	13	14
Length	78 mm.	—	67 mm.	47 mm.	—	—	73 mm.	64 mm.	—
Breadth	—	300	335	—	180	220	330	285	270
Oesophagus	—	—	1170	—	—	—	950	980	—
*Vulva	—	—	910	—	—	—	920	865	—
Tail	—	197	180	—	218	—	275	252	—
Head diam.	—	—	85	—	—	—	93	80	—
Habitat	Heart & hepatic vessels			Heart & post. vena cava			Heart	Heart	
Locality	Vavunikulam, N. Province			Moonemalgaswewa, N.W. Prov.			A'pura N.C.P.	A'pura N.C.P.	
Remarks	Incomplete and damaged Mf. *Brugia* absent *Micipsella* sp. also present			Incomplete and damaged Mf. *Brugia* absent			Complete Mf. *Brugia* absent	Complete and incomp. Mf. *Brugia* present	

* Distance from anterior extremity.
All measurements in μ unless otherwise stated.

1. Hare No. 2 had adults and microfilariae of *Micipsella* sp. only.
2. Hares No. 4 and 10 were negative.

TABLE II

FEMALES (Mature)						
Hare No.	3	3	3	3	3	8
Specimen No.	4	5	6	7	8	15
Length	87mm.	87 mm.	93 mm.	94 mm.	—	82 mm.
Breadth	350	310	350	350	330	310
Oesophagus	1150	980	950	1170	—	950
*Vulva	970	820	1000	1140	—	840
Tail	250	180	230	235	—	220
Head diam.	70	62	78	88	—	80
Habitat	Heart and hepatic vessels					Heart
Locality	Mundumurippu, N. Province of Ceylon					Anuradhapura N.C.P.
Remarks	Mf. *Brugia*++ : Mf. *Micipsella* scanty ; dead remnants of *Micipsella* (?) in hepatic blood clot					Mf. *Brugia*+

* Distance from anterior extremity.

All measurements in μ unless otherwise stated.

1. Hare No. 2 had adults and microfilariae of *Micipsella* sp. only.
2. Hares No. 4 and 10 were negative.

Table III

Males								
Hare No.	3	3	3	3	3	9	9	9
Specimen No.	1	2	3	4	5	6	7	8
Length	45 mm.	41 mm.	50 mm.	—	—	—	—	—
Breadth	170	180	225	—	210	180	185	180
Oesophagus	775	770	–	—	—	—	—	—
L. spicule	285	267	280	275	—	271	—	—
R. spicule	150	127	140	146	—	150	—	—
Spicule ratio	1·9 : 1	2·1 : 1	2 : 1	1·9 : 1	—	1·8 : 1	—	—
Tail	220	185	210	200	—	230	—	—
Head diam.	65	55	80	—	80	—	70	75
Habitat	Heart and hepatic blood vessels					Heart		
Locality	Mundumurippu, N. Province, Ceylon					Anuradhapura, N.C. Province		
Remarks	Mf. *Brugia* ++ in blood; Mf. *Micipsella* scanty; dead remnants of *Micipsella* in hepatic blood clot.					Mf. *Brugia*+		

All measurements in μ unless otherwise stated.

1. Hare No. 2 had adults and microfilariae of *Micipsella* sp. only.
2. Hares No. 4 and 10 were negative.

TABLE IV

	B. buckleyi n.sp. From hare	*B. patei* (Buckley, Nelson and Heisch, 1958) (After Buckley *et al.* 1958). From dog	From cat	*B. malayi* (?) of Buckley and Edeson, 1956 (After Buckley *et al.* 1958) From monkey	From cat	*B. pahangi* (Buckley and Edeson, 1956) (After Buckley *et al.* 1958) From dog and cat
FEMALES	Avg. of 5 ♀♀	Avg. of 6 ♀♀	Avg. of 4 ♀♀	—	—	—
Length	88·6 mm.	43 mm.	39 mm.	48·2 mm.	—	40 mm.
Breadth	334	170	158	148	135	100
Oesophagus	1040	1010	1022	1073	1178	817
*Vulva	954	753	757	690	570	470
Tail	223	198	180	185	—	147
Head	75.6	41·5	40	36	—	36
MALES	Avg. of 3 ♂♂	Avg. of 6 ♂♂	Avg. of 4 ♂♂	—	—	—
Length	45·3 mm.	22·3 mm.	18·9 mm.	18·3 mm.	22·3 mm.	15·1 mm.
Breadth	191·7	93	75	75	80	75
Oesophagus	772·5 (2 specimens)	935	888	944	900	852
L. spicule	274	283	270	342	393	208
R. spicule	139	119	116	106	123	83
Spicule ratio	1·97 : 1	2·4 : 1	2·25 : 1	3·3 : 1	3·1 : 1	2·5 : 1
Tail	205	159	158	142	153	121
Head	66·7	34	33	28	—	24

* Distance from anterior extremity.

All measurements in μ unless otherwise indicated.

The microfilaria while showing the typical tail nuclei, sheath and other important landmarks cannot at present be distinguished from the microfilaria of the other species except perhaps that of *B. malayi* which has a much longer head space.

The habitat of the adult worms in the blood vascular system rather than in the lymphatic system calls for special mention and it is possible that in the single report of *Brugia* type microfilariae in the Giant Squirrel in Malaya by Laing *et. al.* (1960), the adults may have been in the heart or blood vessels. The presence of filarioid worms in the blood vascular system of hares is not an unusual finding, for *Micipsella indica* has been reported from the hepatic vessels of the hare in India by Rao (1936).

The finding of these filarioid worms in the hare suggests interesting applications for the future, particularly if it becomes possible to infect the domestic rabbit with *B. buckleyi*. It will then be most valuable as research material. It will also be interesting to see whether other species of *Brugia* could be transmitted to the domestic rabbit.

Summary

1. A new species of *Brugia* (*Brugiella* subgen. nov.) *buckleyi* is described from the heart and blood vessels of the Ceylon Hare (*Lepus nigricollis singhala*).

2. This worm is much larger than the other species of *Brugia*. It lacks a distinct head bulb and has a relatively much shorter oesophagus. The vulva in the female is close to the posterior extremity of the oesophagus. In the male the caudal papillae differ in their number and arrangement and the spicule ratio is much smaller.

Acknowledgments

We are indeed grateful to Professor J. J. C. Buckley for all the help he has given. We wish to express our thanks also to Dr. D. V. Arndt, Mr. K. Alagarajah, Mr. S. Tharmarasa and Mr. S. Nagamuttu for shooting the hares for us; and to Mr. A. U. Jayaweera for the photographs that illustrate this paper.

References

Buckley, J. J. C., 1960.—"On *Brugia* gen. nov. for *Wuchereria* spp. of the '*malayi*' Group". *Ann. trop. Med. Parasit.*, **54,** 75–77. (W.L. 1063)

———— and Edeson, J. F. B., 1956.—"On the adult morphology of *Wuchereria* sp. (*malayi*?) from a monkey (*Macaca irus*) and from cats in Malaya, and on *Wuchereria pahangi* n.sp. from a dog and a cat." *J. Helminth.*, **30,** 1–20. (W.L. 11224c)

————, Nelson, G. S. and Heisch, R. B., 1958.—"On *Wuchereria patei* n.sp. from the Lymphatics of Cats, Dogs and Genet Cats on Pate Island, Kenya." *Ibid.*, **32,** 73–80. (W.L. 11224c)

Dissanaike, A. S. and Paramananthan, D. C., 1961.—"*Brugia* type adults and microfilariae in a Ceylon Hare." *Trans. R. Soc. trop. Med. Hyg.*, (Correspondence), **55,** 299. (W.L. 21671)

Laing, A. B. G., Edeson, J. F. B. and Wharton, R. H., 1960.—"Studies on Filariasis in Malaya : the vertebrate hosts of *Brugia malayi* and *B. pahangi*." *Ann. trop. Med. Parasit.*, **54,** 92–99. (W.L. 1063)

Rao, M. A. N., 1936.—"*Micipsella indica* n.sp." *Ind. J. vet. Sci.*, **8,** 251–253. (W.L. 9941h)

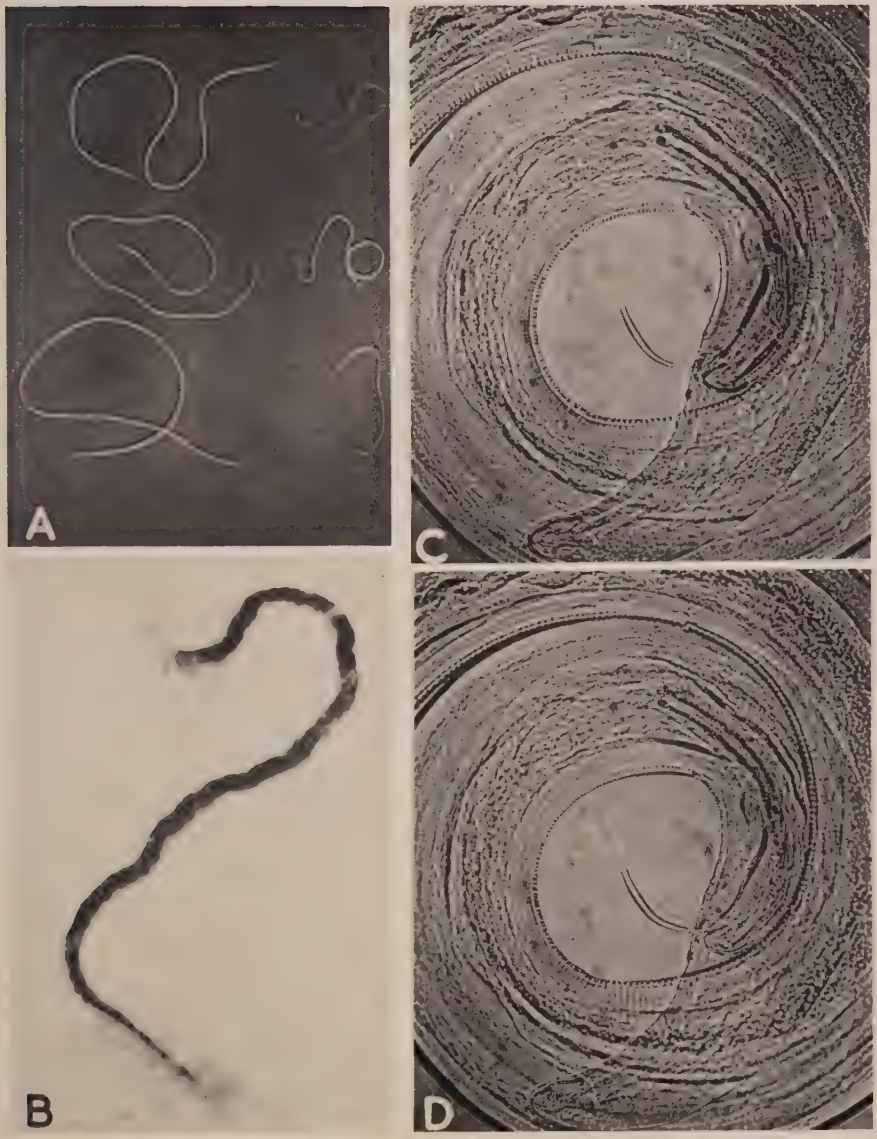


PLATE I

Brugia (*Brugiella*) *buckleyi* n.sp.

Fig. A.—Adult males and females, actual size. Fig. B.—Microfilaria. (X 860). Figs. C and D.—Left lateral view of male tail, photographed at slightly different foci. (X 230).

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 221–224.

On an Unusual Brood Capsule from a Hydatid Cyst of a Goat in Ceylon

By A. S. Dissanaike, B.Sc., M.B.B.S., Ph.D.
and
D. C. Paramananthan, M.A. (Madras)*
Department of Parasitology, Faculty of Medicine, University of Ceylon, Colombo

While examining a large number of hydatid cysts from cattle and goats slaughtered in Colombo, Ceylon, the writers came across an interesting cyst in the lung of a local goat. This cyst appeared quite unusual even before it was incised, for on transillumination it showed a vesicular structure floating within it. The cyst itself was ovoid and measured 2·3 cm. by 1·4 cm., and was placed near the pleural surface of the lung.

On opening the cyst a large number of brood capsules (over 200) was seen (Fig. 1), many attached to the wall (Fig. 2) but several of them floating freely in the cyst fluid. In addition to the latter there was a much larger vesicular structure superficially resembling a daughter cyst, but which on closer examination proved to be merely a modified brood capsule.

Description of the Brood Capsule

It was more or less spherical and much larger than the average brood capsule, measuring 2·9 mm. by 2·7 mm. (Fig. 3). There were 70 scolices all projecting outwards, attached by a pedicle to the outer surface of the brood capsule. Many of the scolices were completely everted as seen in the figure, but a few of them had their head end withdrawn into the body. In order to make quite certain that this was

* Ceylon Association for the Advancement of Science, Research Student.

a brood capsule, a portion of this vesicle was fixed in Bouin's fluid, sectioned at 8 μ thickness and stained in Heidenhain's iron haematoxylin. It was quite clear from these sections (Fig. 4) that we were dealing with a brood capsule and not a daughter cyst as there was no evidence of a laminated layer but only a germinative epithelium with a cuticular lining on the outside. In some sections the continuation of the lumen of this vesicle into the pedicle of the scolex was seen.

Twenty six brood capsules from this particular hydatid cyst were carefully examined and it was found that the number of scolices within them ranged from 5 to 143, the average number being 30. A larger number of brood capsules examined from normal hydatid cysts of cattle and goats collected on previous occasions contained scolices ranging in number from 6 to 87 with an average of 25.

Discussion

It is interesting to compare this brood capsule with a normal one. As described clearly by Cameron (1927), "inside the brood capsules, the scolices develop as a local thickening, which rapidly becomes a small bud on the inner surface of which cuticular material is laid down. The wall tends to evaginate and the papilla continues to develop its typical shape, becomes pedunculated and suckers appear. The cuticular material spreads down the stalk and at the other extremity develops into hooklets. This growth progresses until the mature scolex is formed, swinging on its stalk in the brood capsule It has, however, acquired the faculty of withdrawing its head inside its body in order to protect the important hooks from damage by adiacent scolices." Cameron goes on to say that external budding is never seen and that the so called cases of external development of scolices are due to rupture of the brood capsule which is elastic, tends to turn inside out and so simulate this external budding of text books. One often notices in examining brood capsules that a number of them rupture and collapse in this way and give the impression that the scolices project outwards from a central collapsed mass. In fact Leuckart (1886) mentions that in the normal state these brood capsules never rupture except when exposed to the influence of external agents. It is quite clear therefore, that this particular brood capsule had probably, for some unknown reason, ruptured and collapsed "*in vivo*" and that the ends of the central collapsed mass

of germinal epithelium became united and gradually vesiculated and swelled out to the present size. This is exactly what Coutelen (1927a & b) observed in cultures. As far as the writers are aware this is the first time that this process has been shown to occur in nature. The observation that brood capsules can normally have as many as 87 scolices suggests that after the collapse, reconstitution and swelling out of this particular brood capsule, no fresh budding of scolices occurred. This is further borne out by the fact that all the scolices were at the same stage of development.

One is struck by the close resemblance of this brood capsule to a *coenurus*. In both, the cuticular lining is on the outside and, when fully evaginated, scolices project outwards. A *coenurus*, however, is formed in quite a different manner.

Summary

1. An unusual type of brood capsule is described with the cuticle on the outside and with 70 scolices which are fully developed and projecting outwards. The brood capsule is much larger than the normal brood capsule.

2. This type of brood capsule has been described earlier in cultures by Coutelen (1927) and a similar mode of origin has been suggested for this particular brood capsule.

Acknowledgments

The writers are grateful to Dr. D. W. Amersinghe, Chief Municipal Veterinary Surgeon, Colombo, and to Mr. S. Saravanamuthu, Superintendent of the Slaughter House, Colombo, for providing facilities to collect the material ; and to Mr. P. K. Gomes for the photomicrographs.

References

Cameron, T. W. M., 1927.—"Some Modern Biological Conceptions of Hydatid." *Proc. Roy. soc. Med.*, **20**, 272-283. (W.L. 16915)

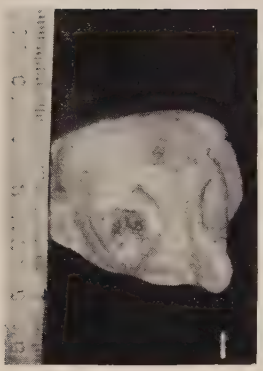
Coutelen, F., 1927a.—"Essai de culture in vitro de scolex et d'hydatides echinococciques." *Ann. Parasit. hum. comp.*, **5**, 1–19. (W.L. 899a)

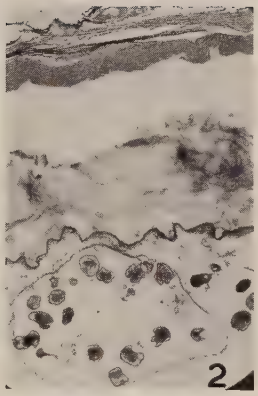
————, 1927b.—"Sur l'evolution vesiculaire in vitro des scolex echinococciques." *Ibid.*, **5**, 239–242.

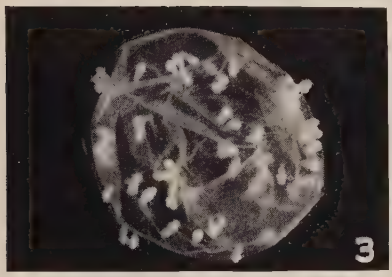
Leuckart, R., 1886.—"Die Parasiten des Menschen und die von ihnen Herruhrenden Krankheiten." *Leipzig und Heidelberg.*

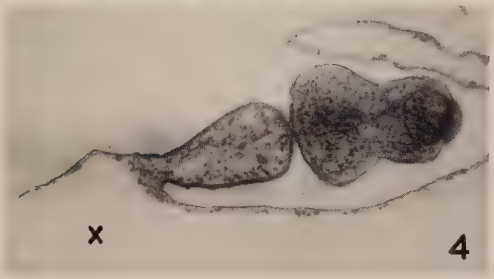
Explanation of Figures

Fig. 1.—Part of lung showing the hydatid cyst. (x $\frac{3}{4}$). Fig. 2.—Section of wall of above cyst showing brood capsules, germinal epithelium and laminated layer. (x 36). Fig. 3.—The unusual brood capsule with scolices projecting outwards, photographed by incident light. (x 13). Fig. 4.—Section of the above brood capsule showing cuticle on the outside and an everted scolex projecting outwards. "x" represents lumen of brood capsule. (x 206).




2


3


x
4

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 225–232.

A Preliminary Note on the Bacteriostatic Properties of Some Helminths of Animals

By H. M. Gharib

Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Cairo

The first two larval stages in the life cycle of strongyloid nematodes have a free living existence. During this time the larva which hatches from the egg feeds actively, undergoes two moults and grows considerably before reaching the infective stage. Under natural conditions this internal development takes place in the faeces, which have been deposited by the infected host on ground likely to be contaminated with various bacteria.

In the laboratory McCoy (1929) succeeded in hatching eggs of *Ancylostoma caninum* in agar cultures of 22 different micro-organisms, and the larvae developed to the infective stage. Gharib (1953) noticed that a species of the free living nematode, *Rhabditis*, was growing and developing for four weeks in a pure culture of *Escherichia coli* and all stages of development could be seen in the plate of culture. Lapage (1933) has pointed out that infective larvae reared on cultures of *B. coli* may contain, in their intestines, bacteria picked up from these cultures before they have become ensheathed. Kawanishi (1929) recovered various micro-organisms from the surface of *Ancylostoma* larvae. Shope (1941) demonstrated that the pig lungworms *Metastrongylus elongatus* and *Chocrostrongylus pudendotectus* were capable of harbouring swine influenza virus and of transmitting it. Gharib (1955) has recovered different strains of micro-organisms from the body surface of infective larvae of the parasite *Nippostrongylus brasiliensis*. Lapage (1937) found that the free living larvae of *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus* spp., *Chabertia ovina*, *Strongylus* spp. from horses, *Graphidium strigosum* and *Trichostrongylus retortaeformis* from the wild rabbit will all develop normally on a strain of *B. coli* recovered by him from the intestines of the infective trichostrongyle larvae reared on faeces. Jettmar (1952) tested the bacteriostatic properties of different organs of *Ascaris megalocephala*. He found that the pericoel possesses the higher titre and that suspensions of the cuticle of the adult worm also inhibit growth of a number of Gram positive micro-organisms. These bacteriostatic substances (Ascaridin) possess a considerable thermostability.

The aim of this investigation is to determine whether parasites possess some bacteriostatic property enabling them to live and feed in surroundings of micro-organisms. All things being equal this property, if proved to be present, might be of value in the rôle played by parasitic helminths in bacterial diseases.

Material and Methods

I. Material. Parasites used in this work were collected freshly from Cairo slaughter houses and comprised the following material :

(a) Adult liver fluke (*Fasciola gigantica*) which were collected from livers of recently killed buffaloes.

(b) *Ascaris vitulorum* which were collected from the intestinal tract of young buffaloes.

The parasites were washed in a sieve under running water for 15 minutes and then washed five times in sterile normal saline solution ; they were then left to drain, and weighed either whole or in parts only. Material after weighing was ground in a mortar with sterile sand until a good paste was obtained, and a known amount of sterile normal saline solution was added. The addition of saline was found necessary, especially in the case of *F. gigantica*, to facilitate filtration. The suspension (parasite extract in saline) was mixed well then passed through gauze, No. 1 filter paper, and through Seitz filter. This procedure was carried out under aseptic conditions as far as possible. The *p*H of the suspension after sterilization (filtrate) was tested and was about 7.5 in *A. vitulorum* and 7·6 in *F. gigantica*. The colour of the filtrate was noticed to be faint yellow in *F. gigantica* and yellowish green in *A. vitulorum*. No bile salts or pigments were present in the filtrate of liver fluke or in the suspension. The bacterial flora of the unfiltered parasite suspension was investigated after culturing on ordinary nutrient agar plates and consisted of :

F. gigantica :

(a) Small colony of *Corynibacteria* (in the bacillary form).

(b) Big colony of Gram positive diplococci and Gram negative rods.

A. vitulorum :

(a) Colony of Gram negative slender rods.

II. Bacteriostatic effect. Several Gram positive and Gram negative micro-organisms were tested. These were : *B. anthracis*, *Micrococcus pyogenes* var. *aureus*, *Pasteurella septica*, *Streptococcus faecalis* and *Escherichia coli*.

A standard loopful of a 1 : 100 dilution of a 24 hours' broth culture at 37°C., of the respective organism, was used as an inoculum.

Five sets of test tubes, of various dilutions of the parasite filtrate in broth, were prepared and each was inoculated with the particular organism. Tubes containing broth only were also inoculated to serve as controls.

Each set consisted of 5 tubes. Tube 1 contained parasite filtrate. Serial dilutions of the parasite filtrate were prepared in serial two fold dilutions in tubes 2, 3 and 4. Tube 5 contained broth only to serve as control. An inoculum of the organism to be tested was added to each of the six tubes.

In order to ascertain any bactericidal action of the parasite, subcultures were made on ordinary nutrient agar from all the seeded tubes after various periods of incubation at 37°C., viz., ½ an hour after the inoculum was added and then after 1 day, 2 days, 3 days, etc. The results are shown in Tables I and II.

Experiment No. 1

Filtrates were prepared from :—

(a) *F. gigantica* (whole parasite) 15 gms. and 100 ml. normal saline.

(b) *A. vitulorum* (whole parasite) 70 gms. and 100 ml. normal saline.

The filtrates of the parasites and control were tested against the respective organisms. Results are shown in Table I.

Experiment No. 2 :

Filtrates were prepared from :

(a) *F. gigantica* (without the anterior and ventral suckers) 15 gms. and 100 ml. normal saline.

(b) *A. vitulorum* (without the mouth parts) 70 gms. and 100 ml. normal saline.

The filtrates of the parasites and control were tested against the respective micro-organism. Results are shown in Table II.

TABLE I

RESULTS OF EXPERIMENT NO. 1

Organism	Duration of exposure		Result of growth in: Fluke filtrate 0	½	¼	⅛	*Ascaris* filtrate 0	½	¼	⅛	Control
B. anthracis	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	2+	4+	4+	4+	4+	4+	4+	4+	4+
	3	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	4	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	5	D	—	4+	4+	4+	3+	3+	4+	4+	4+
Micrococcus pyogenes var. *aureus*	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	4+	4+	4+	4+	—	—	—	3+	4+
	2	D	4+	4+	4+	4+	—	—	—	3+	4+
	3	D	2+	4+	4+	4+	—	—	—	3+	4+
	4	D	1+	4+	4+	4+	—	—	—	3+	4+
	5	D	1+	4+	4+	4+	—	—	—	3+	4+
Streptococcus faecalis	½	H	4+	4+	4+	4+	—	—	—	—	4+
	1	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	3	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	4	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	5	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
Pasteurella septica	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	3	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	4	D	—	4+	4+	4+	4+	4+	4+	4+	4+
	5	D	—	4+	4+	4+	4+	4+	4+	4+	4+
Escherichia coli	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	2	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	3	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	4	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	5	D	5+	5+	4+	4+	5+	5+	4+	4+	4+

Degree of growth as judged by turbidity on broth.

—=No growth. 1+=+ 2+=++ 3+=+++ 4+=++++
5+=+++++ 0=Parasitic filtrate as such H=Hour D=Day.

TABLE II

RESULTS OF EXPERIMENT NO. 2

Organism	Duration of exposure		Result of growth in: Fluke filtrate 0	Fluke filtrate ½	Fluke filtrate ¼	Fluke filtrate ⅛	*Ascaris* filtrate 0	*Ascaris* filtrate ½	*Ascaris* filtrate ¼	*Ascaris* filtrate ⅛	Control
B. anthracis	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	2+	3+	4+	4+	4+	4+	4+	4+	4+
	3	D	2+	3+	4+	4+	4+	4+	4+	4+	4+
	4	D	1+	3+	4+	4+	4+	4+	4+	4+	4+
	5	D	—	2+	4+	4+	4+	4+	4+	4+	4+
Micrococcus pyogenes var. *aureus*	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	2+	3+	4+	4+	—	3+	3+	4+	4+
	2	D	1+	3+	4+	4+	—	3+	4+	4+	4+
	3	D	1+	3+	4+	4+	—	3+	4+	4+	4+
	4	D	1+	3+	4+	4+	—	3+	4+	4+	4+
	5	D	—	3+	4+	4+	—	3+	4+	4+	4+
Streptococcus faecalis	½	H	4+	4+	4+	4+	—	—	—	—	4+
	1	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	3	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	4	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
	5	D	4+	4+	4+	4+	4+	4+	4+	4+	4+
Pasteurella septica	½	H	—	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	2+	4+	4+	4+	4+	4+	4+	4+	4+
	2	D	2+	4+	4+	4+	4+	4+	4+	4+	4+
	3	D	1+	4+	4+	4+	3+	4+	4+	4+	4+
	4	D	—	4+	4+	4+	3+	4+	4+	4+	4+
	5	D	—	4+	4+	4+	3+	4+	4+	4+	4+
Escherichia coli	½	H	4+	4+	4+	4+	4+	4+	4+	4+	4+
	1	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	2	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	3	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	4	D	5+	5+	4+	4+	5+	5+	4+	4+	4+
	5	D	5+	5+	4+	4+	5+	5+	4+	4+	4+

Degree of growth as judged by turbidity on broth.

—=No growth 1+=+ 2+=++ 3+=+++ 4+=++++
5+=+++++ 0=Parasitic filtrate as such H=Hour D=Day.

Discussion

The effect of the parasites on their hosts does not stop at the utilization of food and other necessary materials but they may also mechanically block up ducts, or during the migration of their larvae cause pneumonia or other respiratory disturbances. Another danger from the parasites is that they also play a rôle in the introduction of bacteria and protozoa into the host (Weinberg, 1907 ; Malvoz and Lambient, 1918 ; De Blieck and Baudet, 1920 ; Graybill and Smith, 1920 ; Koyzumi, 1927 ; Kawanishi, 1929 and 1932 ; Tyzzer, 1934 ; Fourie, 1936 ; Podyapolskaya and Dedova, 1937 ; Stein *et al.*, 1939 ; Shope, 1941 ; De Blieck and Jansen, 1944 ; and Gharib, 1957). Several micro-organisms have been recovered from parasites by Lapage (1933) ; Gharib (1955) ; Shope (1941) ; and Kawanishi (1929).

The first two larval stages in the life cycle of some parasitic helminths involves two moults and growth before the infective stage is reached. Under natural conditions this development takes place in the faeces, which have been deposited on ground likely to be contaminated with various bacteria.

Various investigators succeeded in the cultivation of free living larvae on pure cultures of bacteria (McCoy, 1929 and 1929a ; Lapage, 1931 and 1931a ; and Gharib, 1953). The larvae developed only on living bacteria and failed to develop on dead cultures or on bacterial products only (McCoy, 1929a). Lapage (1937) found that larvae can be grown in simple saline solutions to which living bacteria (*B. coli*) were periodically added, so that a relatively very simple medium can be used.

Although this is only a preliminary study, still it can be observed from the results shown in Table I that there is marked bacteriostatic action of *A. vitulorum* filtrate against *Micrococcus pyogenes* var. *aureus* at dilutions of 0.7%, 0·35% and 0·17%. A 0·15% filtrate of *F. gigantica* has a marked bacteriostatic action against *Pasteurella septica*. Both parasites showed some bacteriostatic action against *B. anthracis* in higher dilutions of the filtrate.

The result given in Table II, (using parasites without mouth parts) show that the bacteriostatic action, although present, was not so obvious as in Experiment No. 1.

It is of interest to note that neither of the parasite filtrates proved to have any action against *Streptococcus faecalis* or *Escherichia coli* ; on the contrary the latter organism grew more luxuriantly on the filtrate of both parasites, a finding that agrees with that of Jettmar (1952).

The composition of the medium may be such that the bacteriostatic action is not formed, or if formed is reversibly inactivated. It may also happen that a good medium for the antagonist may not support the growth of the test organism. Most antibiotics have a selective action, and it may well happen that antagonists are not recognized because the particular test organism used is not sufficiently sensitive to the antibiotic. This might explain why *E. coli* grew more luxuriantly on the filtrate of parasites.

Factors other than the bacteriostatic action and the selective media might hinder the growth of the organism; the writer was aware of bile salts and pigments having some inhibitory effect on the growth of some micro-organisms, but the filtrate of the parasites tested were free of them.

It seems that there is some bacteriostatic action possessed by parasites, the nature of which calls for more study. As the parasite grows on bacteria it must possess some bacteriostatic action in order to utilize the bacteria for food. If the bacteria are harmful to the parasite host, is it not possible that they could be equally harmful to the parasite unless the parasite possessed some bacteriostatic action against them?

It would seem that some bacteria are not harmful to parasites, in which case the presence of a bacteriostatic action against the bacteria would be superfluous.

Conclusion and Summary

Studies were made on the bacteriostatic action of the parasites *F. gigantica* and *A. vitulorum* against some animal micro-organisms. From the result of this work, it would appear that both parasites possessed some bacteriostatic action against certain organisms while others had none.

Acknowledgment

The author extends his thanks to Prof. O. Zaki, Head of the Dept. of Bacteriology, Faculty of Veterinary Medicine, Cairo University, for placing at his disposal the facilities of the Department.

References

De Blieck, L. and Baudet, E. A. R. F., 1920.—"Verdere onderzoekingen over de biologie en den infecticweg von de larven von *Strongyloides westeri*". *Tijdschr. Diergeneesk*, **47**, 497. (W.L. 21262)

———— and Jansen, J. 1944.—"De Beteekenis von *Strongyloides westeri* bij de Pyo-Septicaemie von het veulen". *Vlaam. diergeneesk. Tijdsch* **13**, 52–57. (W.L. 22590a)

FOURIE, P. J. J., 1936.—"A contribution to the study of the pathology of Oesophagostomiasis in sheep." *Onderstepoort J. vet. Sci.*, **7**, 277–347. (W.L. 15681a)

GHARIB, H. M. 1953.—"Unpublished work".

————, 1955.—"Some observations on the transmission of bacteria by infective larvae of *Nippostrongylus brasiliensis*". *J. Helminth*, **29**, 27–32. (W.L. 11224a)

————, 1957.—"Review of the literature on the role of tissue invading helminths in bacterial infections." *J. Egypt vet. med. Assoc.*, **3**–36. (W.L. 11172c)

GRAYBILL, H. W. and SMITH, T. 1920.—"Production of fatal blackhead in turkeys by feeding embryonated eggs of *Heterakis papillosa*". *J. exp. Med.*, **31**, 647–655. (W.L. 11189)

JETTMAR, H. M. 1952.—"Über die bakteriostatische Wirkung der Askariden-Cuticula." *Arch. Hyg. Berl.*, **136**, 568–573. (W.L. 1770)

KAWANISHI, K., 1929.—"Experimental studies on the entrance of pathogenic bacilli incidental to percutaneous infection with hookworm." *J. med. Ass. Formosa*, 50–51. (W.L. 11337)

————, 1932.—"Experimental studies on the entrance of bacteria incidental to percutaneous infection of the human body with hookworm." *Ibid.*, **31**, 932–933.

KOIDZUMI, M., 1927.—"A resumé of works on Ascaris". Recently published from the Parasitology Laboratory, Keio University Medical College, *Japan med. World*, **7**, 12–16. (W.L. 10880)

LAPAGE, G., 1933.—"The cultivation of infective nematode larvae on cultures of *Bacillus coli*." *Rep. D. Inst. Anim. Path., Univ. Camb.*, **3**, 237–271. (W.L. 18252d)

————, 1933a.—"Cultivation of Parasitic Nematodes." *Nature, Lond.*, **131**, 583. (W.L. 14900)

————, 1937.—Nematodes Parasitic in Animals. London. 35.

MALVOZ, E. and LAMBIENT, J., 1918.—"Infections microbiennes consecutives à la penetration cutaneé des larves de L'Ankylostome." *Ann. Inst. Pasteur*, **32**, 243. (W.L. 857)

MCCOY, O. R., 1929.—"The suitability of various bacteria as food for hookworm larvae." *Amer. J. Hyg.*, **10**, 140–156. (W.L. 600a)

————, 1929a.—"The growth of hookworm larvae on pure cultures of bacteria." *Science*, **69**, 74–75. (W.L. 19938)

PODYAPOLSKAYA, V. P. and DEDOVA, 1937.—"The interrelation between the infestation by Ascarids and bacterial infections in experimental conditions." Papers on Helminthology published in commemoration of the 30 year Jubileum of K. J. Skrjabin and of the 15th Anniversary of the all Union Institute of Helminthology, Moscow, pp. 491–496.

SHOPE, R. E., 1941.—"The swine lungworm as a reservoir and intermediate host for swine influenza." *Jour. exp. Med.*, **74**, 41–47 and 49–68. (W.L. 11189)

STEIN, C. D., LUCKER, J. T., OSTEEN, O. L. and GOCHENOUR, W. S., 1939.—"Studies on the possible role of endo-parasites in the transmission of infectious anaemia". *J. Amer. vet. med. Ass.*, **95**, 536–541. (W.L. 11022)

TYZZER, E. E., 1934.—"Studies on histomoniasis, or "black-head" infection, in the chicken and turkey." *Proc. Amer. Acad. Arts Sci.*, **69**, 190–264. (W.L. 16599)

WEINBERG, M., 1907.—"Du role des helminths, des larves d'helminths et des larves d'insectes dans la transmission des microbes pathogènes." *Ann. Inst. Pasteur*, **21**, 417–442. (W.L. 857)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 233–254.

On *Skrjabinotaenia cricetomydis* n.sp. (Cestoda: Anoplocephalata) from the Gambian Pouched Rat, Nigeria

By A. R. Hockley
Department of Zoology, Southampton University

During a visit of ten months to University College, Ibadan, Nigeria, a collection of helminth parasites was made from a wide variety of hosts. This paper records 18 specimens of the genus *Skrjabinotaenia* from the Gambian pouched rat, *Cricetomys gambianus* Waterhouse. Five specimens of the host were examined of which three were adults trapped in the field and two half-grown taken alive. The adults were heavily infested by the ectoparasite (commensal?) *Hemimerus* and carried also a considerable number of taeniid worms not yet identified. The two young rats were received more than a week after capture and were quite free from *Hemimerus*. Owing to injury one of them was killed and in the gut 13 specimens of a most unusual tapeworm were found, clearly resembling those ascribed to a new genus, *Meggittina*, by Lynsdale, 1953. The validity of this genus has since been considered by both Canadian and Russian authorities whose views will be discussed below. In the meantime the author accepts *Skrjabinotaenia* as the correct generic name. Lynsdale's material had been long preserved and nothing was known of the living worm nor was the host rat identified. The present material was therefore of interest, increased by the realisation that it formed a new species helping to link *Meggittina baeri* Lynsdale, *Catenotaenia aegyptica* Wolfgang and *Rajotaenia gerbilli* Wertheim, within one group of the family Catenotaeniidae.

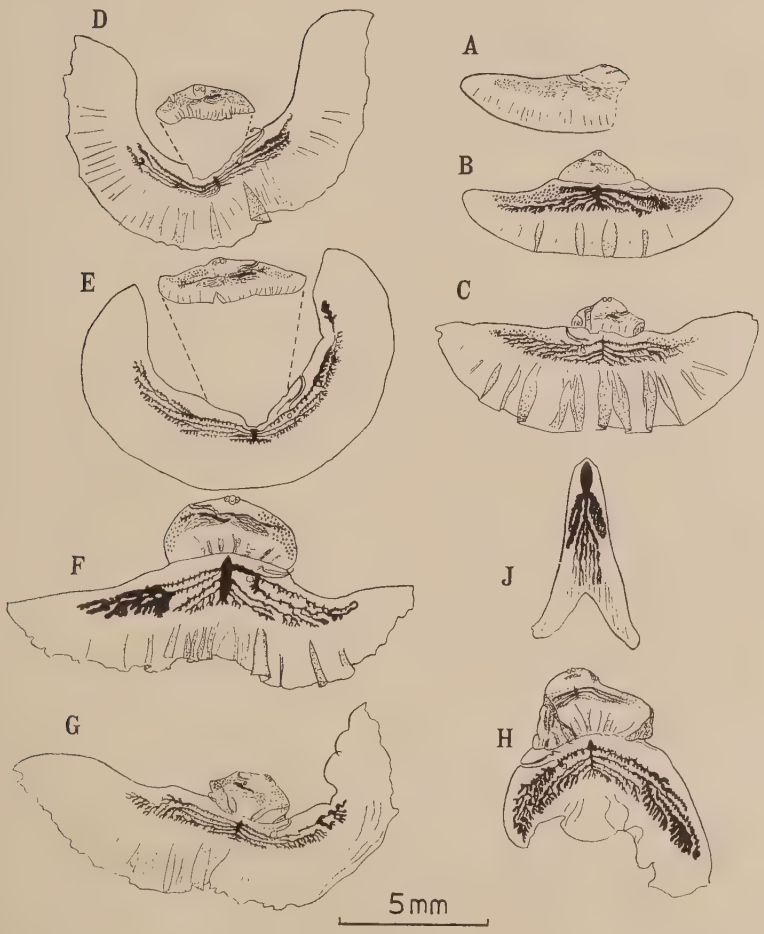
All the worms were in the first half of the duodenum, with the folds of their thin and delicate proglottides closely applied to the mucosa. Gentle irrigation with saline from a pipette soon loosened the hold of the scolex and the worms became much more active, with waves of movement passing outwards along the transversely elongated terminal proglottis and causing the ends to move up and down with a curious resemblance to the wings of a bird. Each worm was composed of only two, or rarely three, proglottides. Close to the 13 attached worms were two detached proglottides shaped in the form of arrow heads, much smaller but thicker than the terminal proglottis of the worm, (Fig. 1, J.). Their movements showed similarities and suggested that they were apolytic proglottides of the same worm, and this was later confirmed from the study of sections.

The circumstances of capture made it probable that the two younger rats were litter-mates, and in the hope that the second was also infected it was kept alive and a daily examination of faeces was made. After 30 days, during which more than 200 proglottides of other taeniid worms were found, a single proglottis of *Skrjabinotaenia*, much folded but quite intact, was found in a faecal pellet, and it was of the size and shape of the normal terminal segment of the worm. No others were released in a further period of 25 days and when the rat was killed five normal *Skrjabinotaenia* were found, all within one inch of the duodenum, with 21 long tapeworms of another type extending throughout the small intestine.

Before fixation the worms were washed with Ringer's solution and carefully observed alive. Warming in distilled water extended and immobilised them without causing any apparent distortion. The earlier specimens were then fixed without pressure using hot Susa fixative. Some folds persisted, specially in the younger proglottides, which were inconvenient for the interpretation of mounted or sectioned specimens, but they gave good staining with Ehrlich's haematoxylin. Later specimens were fixed with 90% alcohol under light pressure from a cover glass, but their staining was inferior. Most of the specimens were brought to cedarwood oil as a clearing agent and the worms then chosen for sectioning were first drawn under a binocular microscope before embedding in paraffin. The others were mounted in canada balsam.

Generally the worms showed a crescentic shape, with the horns of the crescent curved in a forward direction. The range of shapes found is illustrated in Fig. 1. Lynsdale found only one specimen with this direction of curvature and considered it to be atypical. It was quite possible for the living worms to assume the reverse curvature, but generally it involved very pronounced folding of the delicate posterior edge.

Detailed examination of all this material has shown that the present specimens must be assigned to a new species, for while there are many points now emerging which could be additional to Lynsdale's observations there are others which are in direct conflict. The author has had the opportunity to examine two of Lynsdale's specimens in the collection of the London School of Hygiene and Tropical Medicine and is in general agreement with Wolfgang (1956) in their reinterpretation. With regard to the generic name, Wolfgang prefers to unite a whole series of related tapeworms under *Catenotaenia*, but for reasons discussed below the author is more inclined to agree with Spasski (1956) and assign both *Meggittina baeri* and the new worms to *Skrjabinotaenia* Akhumian, 1946.



Skrjabinotaenia cricetomydis n.sp.

Fig. 1.—A series of outline drawings showing the range of shape and the pattern of the gravid uterus (black). A, the youngest in development, is an incomplete specimen. In D, and E, the younger proglottides have been detached to facilitate flattening, the broken lines indicate their true position in the worms. H, shows the greatest development with three proglottides. J, an apolytic proglottis of thickened arrow-head shape.

CATENOTAENIIDAE Spasski, 1950

SKRJABINOTAENIA CRICETOMYDIS n.sp.

The scolex consists of a group of four rounded, unarmed suckers of average diameter 0·14 mm., separated by fairly well defined cuticular grooves ; in the centre of this group is a minute vestige of an apical sucker visible in all the sectioned specimens and in some of those mounted whole. There is no neck, and the first indication of the development of a new proglottis is the appearance of a rudiment of the genital ducts on one side or the other immediately behind one of the suckers. After this a faint line of demarcation appears to separate it from the scolex. In all cases it was possible to determine the position of two sets of genital ducts, and they were found to be on alternate sides in 15 of the 18 worms. Three specimens showed the development of a third segment, and in two of them the ducts were again in the alternate position. In respect of the degree of development the 18 worms could be grouped in four categories, but in these the use of the terms "young" and "older" must be understood to imply not absolute age but rather the degree of development of proglottides shown at the time the animal happened to be fixed.

1. Three specimens were young with only faint rudiments of a second proglottis (Fig. 1, A). In the first (terminal) proglottis the uterus was filled with undeveloped eggs. One of these specimens was exceptional to the general rule in retaining the ovary still visible in the older proglottis. It seems likely that one of the apolytic proglottides (Fig. 1, J) had been recently detached from this specimen.

2. Four specimens were older, with the second proglottis well developed towards maturity but without a gravid uterus. Transverse extension of this proglottis was beginning but the line of attachment to the older proglottis restricted this process, and the younger segment therefore became considerably folded. It was found convenient and easy in worms of this age and older to detach the proglottides in the clearing reagent and mount the younger ones in a more flattened position than would otherwise have been possible. The first proglottis showed the uterus full of young embryos but the embryophores were very slightly developed. No trace of ovary remained in the first proglottis though in most the vitelline gland was just visible (Fig. 1, B, C, D).

3. Eight specimens were older again with a gravid uterus in both the proglottides and showing two stages of egg development (Fig. 4). In the younger segment only the first two branches of the

uterus could be seen in most of the specimens mounted whole (Fig. 1, E, F, G), but sections showed that the others were present.

4. A group of three worms showing the most advanced development also showed indications of a third proglottis (Fig. 1, H). The second was well expanded but deeply folded at its attachment to the terminal proglottis. The uterus showed the full pattern of branches but was still rather small. In the oldest proglottis the uterus reached its full size and the embryophores were well formed round the embryos.

The Reproductive System

Examination of the whole range of proglottides available, both of specimens mounted whole and in sections, made it possible to interpret the whole of the reproductive system with some accuracy. The results are seen in Fig. 2, which is diagrammatic to the extent that the ducts are simplified for a single plane drawing, and the uterus does not in fact show the full development of its branches in a segment still containing an active ovary and oviduct full of eggs. In the female system the vagina is a long curving tube with a narrow lumen and well defined cuticular lining. A sheath of glandular cells surrounds the vagina which is slightly thickened at the proximal end where the lumen is constricted. The length of the cirrus is such that its insertion up to this point in the vagina would be possible. The vagina now leads into a pear-shaped receptaculum seminis. The first branch of the uterus crosses the neck of this vesicle and often appears to cause some distortion or constriction at that point, but since a bipartite receptaculum seminis occurs in other Catenotaeniidae the uterine branch is probably not the cause. The duct leaving the receptaculum seminis on its mesial side has a clearly defined cubical epithelium and is soon joined by a similar duct from the ovary ; the common duct then continues to the region usually known as shell-gland. Here the system is joined by the short neck of the vitelline gland, scarcely perceptible as a separate duct. The vitelline gland is a darkly staining mass in two main lobes joined at the neck and lying close behind the receptaculum seminis. In a section truly transverse to the axis of the worm the vitelline gland shows a mass of lobes roughly in the shape of the letter H, with the neck leaving at the centre. These ducts lying close to the large rounded part of the receptaculum seminis show some twists but the chief difficulty in their observation is the mass of unicellular glands (Mehlis' gland) in which they are embedded, and their relationships have only been worked out with the aid of serial sections. The gland cells open

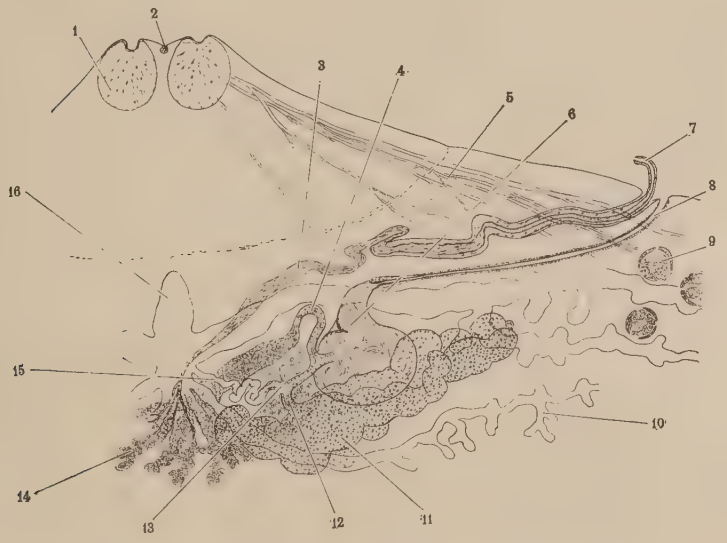
to the shell-gland area of the duct in two main clusters and it seems probable that they contribute to the formation of the delicate membrane that here surrounds the egg and one or two vitelline cells. It is also assumed that fertilisation has taken place along the course of the ducts just described. From this point the duct carrying the completed eggs to the uterus may be called the uterine oviduct. It has a thin but firm wall and is much twisted, and it joins the central stem of the uterus between its first and second branches.

The ovary in *S. cricetomydis* is visible in all the younger proglottides, but it seems that ovulation must occupy a relatively short time and result in the almost complete disappearance of the ovary, for little trace of it can be found in any of the older proglottides, where the uterus is gravid. It is difficult to think of the ovary as having any true wall, although the developing oocytes are placed together in well defined masses, for as ovulation occurs the ovary vanishes altogether with the closing up of the parenchymatous connective tissue.

In the younger mature proglottides the ovary is quite distinctly but irregularly branched and is nearly symmetrical in the centre of the segment. Fig. 2 shows only a very small part of the branches, whose number varies between four and ten on each side. As in the vitelline gland, the ovarian branches seem to be disposed in two layers, and there are commonly two branches less on the side carrying the vitelline gland, i.e. the poral side. It is sometimes held that the vitellarium and germarium are two specialised parts of the primitive platyhelmian ovary, and the vitellarium of *S. cricetomydis* might therefore be homologised with the anterior two branches of the ovary on the poral side. All the other ovarian branches meet centrally and from this point a broad lobe of the organ departs asymmetrically towards the poral side, where it soon narrows to form the true oviduct.

The uterus is particularly clear and characteristic in a gravid proglottis. The central stem is quite distinct and the anterior pocket is prominent as in many Catenotaeniidae. The main lateral branches are usually five in number, decreasing both in size and regularity from the front backwards. Each of these branches carries subbranches along most of its length and mainly from the posterior side, but small sacculations occur opposite to them on the anterior side. The branches also overlap in some cases and may rarely anastomose. The general plan is illustrated in Fig. 3 and some variations may be seen in Fig. 1. The younger proglottides in which the ovary is well developed sometimes show the uterus as slender empty canals in the position of the first two or three branches. The author is not prepared

to say that the other branches are not developed, for they would lie in the region which is occupied by the densely stained ovary and therefore would be difficult to detect, especially in their empty state.



Skrjabinotaenia cricetomydis n.sp.

Fig. **2**.—A diagrammatic reconstruction of the front end of the worm, with parts of the mature reproductive system. **1**, sucker of the scolex ; **2**, vestigeal apical sucker ; **3**, vas deferens ; **4**, oviduct ; **5**, excretory canals ; **6**, receptaculum seminis ; **7**, cirrus ; **8**, vagina ; **9**, testis ; **10**, sub-branch of the uterus ; **11**, vitelline gland ; **12**, vitelline duct ; **13**, shell gland ; **14**, a small portion of the ovary ; **15**, uterine oviduct ; **16**, anterior pocket of the uterine stem.

The male system contains a large number of oval or rounded testes in two antero-lateral fields. This is the thickest part of the proglottis and two or even three testes may overlap so that they are difficult to count. The number is rather smaller on the poral side, 70—89 having been counted but it is possible that 100 could be present ; on the aporal side the range is 74—138. The diameter of a testis varies between 55μ and 80μ. As is usual, no clear vasa efferentia can be seen, and the spermatozoa must be presumed to pass through the spaces of the parenchyma and accumulate in channels which form recognisable tributaries of the vas deferens. Such channels may be seen on either side of the middle line in the

posterior part of the proglottis, joining in the centre to form the vas deferens which proceeds with some enlargement towards the cirrus sac. No doubt it is large enough to store a considerable bulk of spermatozoa, but there is no true seminal vesicle. The duct narrows and describes a sharp loop immediately before entering the cirrus sac. The distal two thirds of the cirrus sac is occupied by a narrow ejaculatory duct, surrounded by some slight glandular tissue, and it is probable that only this narrow tube can be extended within the cirrus on eversion. Several specimens showed the cirrus everted to some length, and in one preparation the cirrus can be seen within the terminal part of the neighbouring vagina, thus demonstrating self impregnation. The cirrus sac lies always immediately in front of the vagina, and the common genital pore is at the extreme front end of the anterolateral margin.

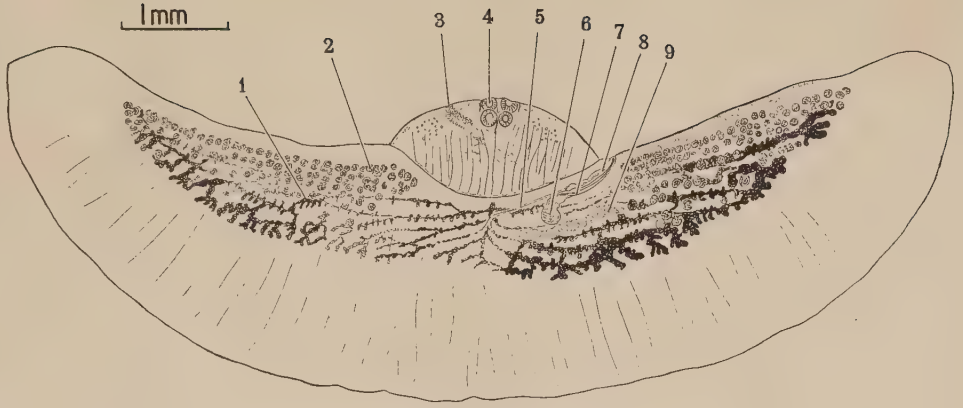
The Excretory and Muscular Systems

Excretory canals can be seen only in parts of the specimens mounted whole, and were not detected with the magnification that could be applied to the living animal. There is apparently a slight plexus of canals in the scolex region from which a group of longitudinal vessels leaves on each side. Branches occur with fair frequency and in a few cases anastomosis of the canals, forming a network, can be seen. Sections show that the canals lie principally in the inner mesenchymal zone, and there is no significant size difference in the canals of the opposite surfaces. Flame cells have not been detected.

Muscles are formed in the usual subcuticular position and a deeper band, mainly of longitudinal fibres, separates the mesenchyme into medullary and cortical zones. In sections of the expanded proglottides there is little sign of dorso-ventral muscles, but they are prominent in the contracted apolytic proglottides. It is probably the contraction of the subcuticular muscles that throws the surface into a series of smooth ridges separated by slight but clear cuticular grooves in the longitudinal direction. In the living animal this enhances the resemblance to a bird's wing by simulating the primary and secondary feathers.

Development of the Eggs

With the range of specimens available it was possible to examine sections of the ovary and uterus at various stages of development. The ovary appeared as a mass of rather large cells staining more deeply than the surrounding parenchyma, and as the ova grew in size they became closely pressed together in the branches of the organ.



Skrjabinotaenia cricetomydis n.sp.

Fig. **3**.—A detailed drawing of one of the younger specimens, showing the separation of testicular fields by the female system. 1, a main branch of the uterus; 2, testis; 3, genital pore of the younger proglottis; 4, sucker; 5, vas deferens; 6, receptaculum seminis; 7, vagina; 8, cirrus sac; 9, vitelline gland.

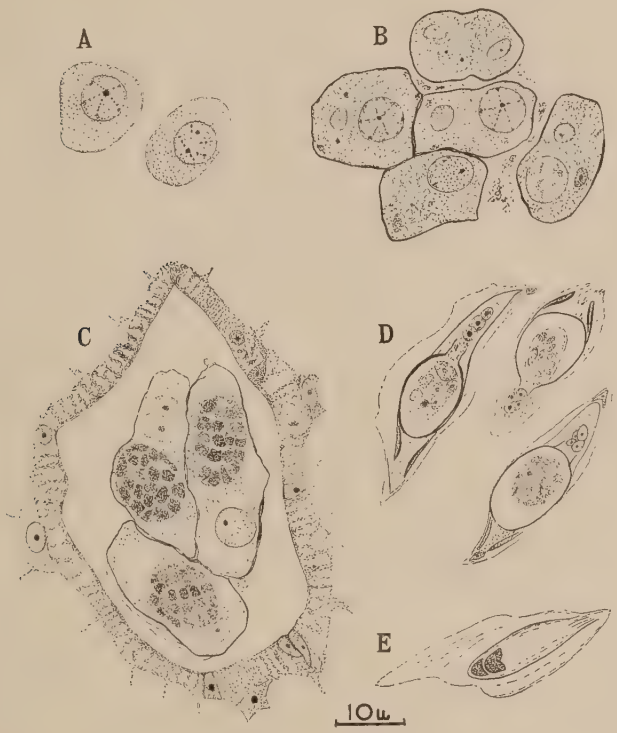
At no stage was a wall detected and on ovulation the organ simply disappeared. Ripe eggs entering the oviduct were 8μ or 9μ in diameter, (Fig. 4, A). Their cytoplasm was uniformly granular and the large vesicular nucleus tended to show a radial pattern of chromatin.

Sections of the younger proglottis in most cases showed the uterus filled with undivided eggs, each accompanied by one or two vitelline cells and enclosed in the shell membrane (Fig. 4, B). Although spermatozoa or male pronuclei are to be expected at this stage it was not possible confidently to identify them. The shell membrane is quite distinct at this stage but shows a considerable irregularity in thickness. Slightly older proglottides showed enlargement of the eggs by stretching of the shell membrane and cleavage of the cells within it, reaching 24μ by 11μ. Fig. 4, C shows a very small section of the uterus containing three eggs. The embryo is a morula of darker stained nuclei, while the vitelline or nurse cells are slightly enlarged and show division only in one of the eggs. Differences between the cells of the morula are not sufficiently consistent to allow the distinction of macromeres, mesomeres and micromeres. There is no secondary membrane or embryophore at this stage. The transition from the stage seen in Fig. 4, C to 4, D is difficult to interpret but it is clear that the embryo is becoming enclosed in a second and thicker protective membrane or embryophore. A group of four granular oval cells seems clearly to be involved in the formation of the embryophore and the morula of cells within it seems smaller than in the previous stage. This suggests that the cells forming the embryophore are blastomeres and not yolk cells. A possible trace of the yolk cell or cells remains at the extreme tips of the elongated egg at this stage. The whole egg, taken from the apolytic proglottis (Fig. 4, E) may be described as a spindle shaped structure with rather irregular wing- or blade-like extensions. At this stage the egg is rather resistant to dehydration by alcohol and little significance should be attached to the shape of the embryo shown. Fig. 4, D strongly suggests that the embryophore is specialised in *S. cricetomydis* with the formation of at least a rudimentary pyriform apparatus, and at this stage the eggs measure about 30μ by 12μ.

Discussion

The present specimens of *S. cricetomydis* are of interest in two ways, firstly for the observations made upon the worms and host in a living state and secondly for the position of the species in systematic classification.

Direct observation of the living worms showed the range of shapes that they could assume. This is illustrated in Fig. 1, though the figure cannot explain the strikingly wing-like movements of the



Skrjabinotaenia cricetomydis n.sp.

Fig. 4.—Development of the eggs. A, ripe eggs in the ovary; B, a group of five uterine eggs with vitelline cells enclosed in the shell membranes; C, a small section of the uterus containing three older eggs with embryos; D, three mature eggs sectioned in the uterus, showing the embryophore and rudimentary pyriform apparatus; E, a whole egg from the apolytic proglottis.

transversely elongated proglottis. The crescent curving forwards was the more usual and relaxed position, but it is unlikely that a reliable difference in this respect exists between the species. The demonstration of apolytic proglottides is new, and particularly the

sharp change of shape noted in two of the specimens. The failure of the captive rat to produce this shape of proglottis is not easily explained. There was good reason to think that the two young rats were infected at similar times, yet 30 days elapsed before any of the five worms present produced an apolytic proglottis and no more were found in a further 25 days. The proglottis was passed out without any of that modification by contraction seen in the first two. The contraction involved the cuticle in a fine series of close folds, giving a superficial appearance of a much thicker state, but both the histology and the study of eggs amply confirmed the relationship between these two proglottides and the mature strobila. In captivity the *Cricetomys* was fed on bread and banana with occasionally a few ground-nuts which were not readily taken. To reduce the work of faecal examination no excess of food was allowed, but the rat appeared to be satisfied and showed some growth, and also the other tapeworms present showed a high rate of apolysis. The number of faecal pellets produced per day (25–30) was probably well below that of the natural state, but it is difficult to appreciate why this should have altered the pattern of apolysis in *Skrjabinotaenia* alone. It is at least an indication that *S. cricetomydis* has a low rate of growth.

The host rat, *Cricetomys gambianus* Waterhouse, occurs widely throughout Africa south of the Sahara, with several varieties in Nigeria alone. Rosvear (1950) expressed doubts about the validity of these varieties, and the two young specimens could not have been reliably identified to any one of them. The rat readily frequents houses and granaries and could have been the host of *S. baeri* in Rhodesia.

As a routine measure the host rats were carefully examined for ectoparasites or commensals, but none could be found. *Hemimerus* sp. is invariably associated with *C. gambianus* in a wild state, but in captivity they are rapidly lost. In view of a possible association with the tapeworm attempts were made to establish a few *Hemimerus* on the captive host. Although they were very active the rat ignored them, but they repeatedly fell off through the wire mesh of the cage that was needed to collect faeces. It seems possible that in the natural burrow these insects may frequently leave the host and could ingest the tapeworm eggs with faecal matter. It is regrettable that no progress could be made in tracing the life history, either through associated insects or mites, but the description of the developing eggs suggests that a specialised embryophore may be formed with a structure resembling the pyriform apparatus of some Anoplocephalidae.

The features described above leave no room for doubt that the new species is closely allied to *Meggittina baeri* Lynsdale, *Catenotaenia aegyptica* Wolfgang and *Rajotaenia gerbilli* Wertheim, and that all of these are true members of the family Catenotaeniidae, if this group is to be given family rank (Spasski 1951, Wardle and McLeod 1952) and not retained within the family Anoplocephalidae Fuhrmann, 1932. Wolfgang (1956) prefers to recognise the Catenotaeniinae only as a sub-family of Anoplocephalidae. Without becoming involved in systematic discussions at this level, it is important to consider whether all the genera proposed are warranted. *Meggittina baeri* Lynsdale, 1953 has been assigned to *Catenotaenia* by Wolfgang; both he and the present author have examined Lynsdale's type specimens preserved at the London School of Hygiene and Tropical Medicine, and we are agreed in reinterpreting the material as follows.

(1) The scolex includes a small apical organ or sucker.

(2) The uterus has a single lateral branch or extension on each side.

(3) The testicular fields are more properly described as lateral, but there is no suggestion of any extension posterior to the ovary.

Detail of the reproductive and excretory systems is very difficult to see in these specimens, but on comparing them with the new material of *S. cricetomydis* the present author is convinced that the ovary and vitelline gland have been wrongly interpreted. The ovary is visible only in the younger segment and is a dendritic structure approximately central in the segment. The vitelline gland is a large lobed mass lying close behind the receptaculum seminis, and therefore displaced towards the poral side. The union of these organs with the receptaculum seminis and with the uterus cannot be interpreted with accuracy, but nothing has been detected that is incompatible with the description here given for *S. cricetomydis*. The branching of the uterus is again difficult to follow, in great contrast to *S. cricetomydis*. Wolfgang has stated (p.17) that in *C. baeri*, with only a single lateral branch on either side of the stem, the branches are like the voluminous branches of *C. oranensis* and *aegyptica*. His Fig. 16 supports this, but later (p. 19) he describes the secondary branches in *C. aegpytica* . . . "which tend to overlap with the branches contiguous to them and give an appearance of anastomosing, although actually each branch is distinct from the others. This dendritic condition is repeated in *C. baeri* and *C. oranensis*." Lynsdale does not describe a dendritic condition, and the present author considers the term unjustified for her species.

In the lateral part of the segment there is a confused mass of short lobes or branches containing the eggs ; a condition rather different from *C. oranensis*, though perhaps derivable from voluminous branches of the type seen in *C. aegyptica*.

Rajotaenia gerbilli Wertheim, 1954 must now be examined for its relationship to *Catenotaenia*. This was briefly considered by Wolfgang on the published description alone, but the present author has examined three paratypes in the British Museum (Nat. Hist.) and is satisfied that, as in *C. baeri*, the specimens amply justify inclusion in the same genus as *C. aegyptica*. The following features are relevant :—

(a) A vestige of an apical organ or sucker is traceable in two specimens, though less distinct than in either *C. baeri* or *S. cricetomydis*.

(b) The shape of the worm and number of segments is very similar to, and intermediate between, *C. aegyptica* and *S. cricetomydis*.

(c) The cirrus pouch is not entirely absent, but it is reduced and less conspicuous than in the other species.

(d) The structure described as germo-vitellarium is in fact the vitelline gland. In gravid segments of *R. gerbilli* it is difficult to distinguish but its disappearance is no more complete than in the other species. The ovary is a central dendritic structure visible only in quite young proglottides.

(e) Some anastomosis does seem to occur between overlapping branches of the uterus, as also is seen in *S. cricetomydis*, but it is not on such a scale as to warrant comparison with a net-like uterus.

(f) The uterine eggs show distinct resemblances to those of *S. cricetomydis*, and with a possible pyriform apparatus they are in keeping with what is known of some anoplocephalid eggs, but this has not been seen in other *Catenotaenia*. The nearest approach to this structure is reported in *C. chabaudi* Dollfus, 1954. The present series of specimens of *S. cricetomydis* shows clearly that a fully gravid segment may easily be described with eggs in an early stage of development, and evidence of the existence of a complex embryophore may then be lacking. This is particularly likely when apolytic proglottides have not been found. Until the development and life history of more of the Catenotaeniidae is revealed it is unwise to attach generic significance to apparent differences in the eggs.

(g) The excretory system of *R. gerbilli* is described as consisting of 20 narrow longitudinal vessels distributed over the width of the worm and anastomosing. The present author found these difficult to distinguish in the three *R. gerbilli* available, but an essentially similar system was seen in *S. cricetomydis*. None could be seen in *C. baeri*, yet Wolfgang asserts that this species and *C. aegyptica* and *C. oranensis* have simple lateral excretory vessels. Until reliably detailed descriptions of the excretory ducts are made they can have little real use in systematic arrangements.

Yamaguti (1960) records that Spasski (1956) considered the genus *Rajotaenia* to be insufficiently determined from *Skrjabinotaenia* Akhumian, 1946, but Yamaguti himself not only retains the genus but makes from it a new subfamily Rajotaeniinae. The history of *Skrjabinotaenia* is involved and tends to add confusion. In certain anoplocephalid tapeworms the young uterus breaks down into a number of capsules in which the eggs become dispersed throughout the parenchyma of the gravid segment. This feature led Meggitt and Subramanian (1927) to transfer *Catenotaenia symmetrica* Baylis, 1927 to *Oochoristica*. Apparently in ignorance of this change Akhumian (1946) was led by similar observations to transfer *C. symmetrica* to *Mathevotaenia* n.gen. Furthermore he claimed that a similar breakdown of the mature uterus into capsules occurred in *C. oranensis*. He used this character, together with the asymmetrical position of the female organs and the restriction of testes to the lateral fields, to justify a new genus *Skrjabinotaenia* for the species *C. oranensis*. These two new genera he placed in a new sub-family Mathevotaeniinae. This behaviour of the uterus in *C. oranensis* is at such variance with previous descriptions that doubt is thrown upon the correctness of Akhumian's identification of this species, and the new sub-family has little value. He received some slight support from Tenora (1959), who also separated *C. oranensis* to form the genus *Skrjabinotaenia*. Here the gravid uterus is shown with a short median stem from which dendritic branches arise only from the posterior end, while previously a much more typical catenotaeniid condition has been shown with some lateral branches, though the more posterior of these are the larger.

Tenora further complicated the subject by placing all other members of the Catenotaeniidae in the genus *Catenotaenia*, and distributing them among three sub-genera as follows :—

1. Sub-genus *Catenotaenia* s. str., with testes behind the other

genital organs ; including :—

Type : *C. pusilla* (Goeze, 1782) Janicki, 1904 ; *C. dendritica* (Goeze, 1782) Janicki, 1904 ; *C. linsdalei* McIntosh, 1941 ; *C. cricetorum* Kirschenblatt, 1949 ; *C. cricetorum* forma *glareolica* Tenora, 1959 ; *C. rhombomydis* Schulz and Landa, 1934 ; *C. geosciuri* Ortlepp, 1938.

2. Sub-genus *Spasskijela* ; with testes sometimes also on each side of the female organs ; including : *C. lobata* Baer, 1925 ; *C.* (*S*). *kratochvili* Tenora, 1959 ; *C. capensis* Ortlepp, 1938.

3. Sub-genus *Meggittina* (Lynsdale, 1953), with testes in front of the female organs and reaching out to the sides.
C. (*M*). *baeri* (Lynsdale, 1953).

Tenora seems to have been unaware of the work of Wolfgang (1956), Wertheim (1954), or Joyeux and Baer (1945), and the present author finds little merit in his arrangement.

Joyeux and Baer (1945) have summarised in tabular form all the recorded variations in the species of *Catenotaenia*. Wolfgang (1956) added the data for more recent species that he assigned to the genus, and in his table he distributed all these species between two groups whose distinguishing characters may be summarised as follows :—

TABLE I

Criteria for the grouping of species of *Catenotaenia*, modified from Wolfgang, 1956.

		Group I *dendritica* type	Group II *oranensis* type
a.	Position of genital pore	In anterior half of segment	At anterior end of the lateral margin
b.	Position of testes	Behind ovary	In two distinct fields lateral to ovary but may be united behind it
c.	Uterine stem	Long	Shorter
d.	Number of uterine branches per side	Greater than 10	Less than 10
e.	Shape of gravid segment	Longer	Broader

At this point it is perhaps necessary to remark that the usual method of counting the uterine branches takes the number of branches at their origin from the main stem. Since the number varies, with slightly fewer on the poral side, it is convenient to show the range as for example : *C. dendritica* (Goeze, 1782) 30–45 × 2. It is easier and more reliable to count the branches where they depart from the main stem rather than to take the number of sub-branches where they approach the lateral margin of the proglottis, for these final branches of the system are far more influenced by pressure and by the number of eggs that they contain. Differences in this practice may have led to *C. lobata* being recorded with 12–20 branches (Baer, 1925) and 10–15 branches (Wardle and McLeod, 1952). Wolfgang (1956) quotes the higher number, but his Fig. 16 shows only 9–10 primary branches for *C. lobata*.

On the criteria given in Table I the *dendritica* Group I includes the following species :—

C. dendritica (Goeze, 1782) Janicki, 1904.
syn. *C. linsdalei* McIntosh, 1941.
C. ris Yamaguti, 1942.
C. laguri Smith, 1954.
C. peromysci Smith, 1954.
C. pusilla (Goeze, 1782) Janicki, 1904.
C. rhombomydis Schulz and Landa, 1934.
C. geosciuri Ortlepp, 1938.
C. cricetorum Kirschenblatt, 1949.
C. reggiae Rausch, 1951.
C. californica Dowell, 1952.
C. chabaudi Dollfus, 1953.

For the species within the *oranensis* Group II separate generic rank is now suggested, and it is clear that the name *Skrjabinotaenia* must have preference even if we are not in full agreement with Akhumian and Tenora in the diagnosis of the genus. The species that fall clearly in this genus are as follows :—

S. oranensis (Joyeux and Foley, 1930).
S. aegyptica (Wolfgang, 1956).
S. baeri (Linsdale, 1953).
S. gerbilli (Wertheim, 1954).
S. cricetomydis n.sp.

A few species remain whose placing must be reconsidered when further information becomes available. Wolfgang has placed *C. lobata* in the second group, and it is possible that he was influenced by an indirect quotation of Akhumian (1946). He writes : "Akhumian (according to Dollfus) has split the genus *Catenotaenia* into two genera, *Catenotaenia* and *Skrjabinotaenia*. The latter was created for *C. oranensis* and *C. lobata* primarily on the basis of the testes being distributed in two fields lateral to the ovary". But this is not true for Akhumian is quite explicit on the relationship of *C. lobata* when he writes "Therefore *C. dendritica*, *C. lobata* and *C. rhombomydis* must be considered undoubted representatives of the genus *Catenotaenia*". Wolfgang himself sees the difficulty when he notes that the two lateral testicular fields are joined posterior to the ovary in *C. lobata*, and his Fig. 16 shows the gravid segment with the uterine stem occupying the whole length of the proglottis. Accepting the fact that this proglottis is a little shorter than is usual in the *dendritica* group, the uterine stem is full length and could not well be longer. The author has not seen any material of *C. lobata*, and since the genital pore is figured at the anterior end of the lateral margin he must accept that the position of *C. lobata* in this grouping is uncertain.

Tenora has placed *C. lobata* and *C.* (*S*). *kratochvili* in the subgenus *Spasskijela*, together with *C. capensis* Ortlepp, 1940. The uterus in *C. capensis* appears to be very different from most other species. According to the figure given by Tenora (after Erhardova and Rysavy) the uterus shows an irregular pattern of branches with no central stem at all, yet Erhardova and Rysavy (1955) have made the species synonymous with *C. lobata* and transferred both to *Skrjabinotaenia*.

A worm similar in some respects to *S. baeri* and *S. cricetomydis* was described as *Eurycestus avoceti* Clark, 1954. Too little is yet known to assign a clear position for this genus, and it seems doubtful if Clark has seen a fully gravid uterus. He placed it in the Cyclophyllidea and has sought its relations among others of the family that are also possessed of two sets of reproductive organs per segment. This is an unprofitable approach, for Baer (1951) has shown that the tendency to reduplicate these organs in each segment has occurred repeatedly, listing no less than twenty genera in pairs which differ only in this respect. He noted that most of these cases occur among the Anoplocephala, and it is here that the affinities of *Eurycestus* should probably be sought. The short and very broad strobila of 9–15 segments, form of vagina and receptaculum seminis and the vitelline gland all resemble *Skrjabinotaenia*. The scolex is much

more reduced and the host was the avocet, but all the Catenotaeniidae are found in rodents. Baer noted that in all his recorded cases genera with single and with reduplicated organs both occupy closely allied hosts, and the presence of *Eurycestus avoceti* in a bird is alone sufficient to suggest only a remote relationship to *Skrjabinotaenia*.

Spasski (1951) has commented on the influence of quickness of formation and speed of ripening of segments upon the length of the cestode strobila. A high rate of production but slow ripening of the proglottides would clearly produce a long strobila, while the reverse situation could account for the proportions seen in *Skrjabinotaenia*. If separate gradients exist in these two functions, whether genetically or envlronmentally determined, it is possible to visualise the evolution of a series such as is found in Catenotaeniidae. The resemblance between *Eurycestus* and *Skrjabinotaenia* in this case could be due to similar variation in the growth pattern rather than to a genetic relationship.

In conclusion, the author has no claim to specialist experience in the field of cestodology, but with 25 years experience of general zoology he is firmly opposed to the creation of sub-genera and genera on insufficient foundations. A host may yield a small number of specimens, for which a complete description including life-history cannot be given, but whose morphology seems to justify exclusion from any known species. With limited knowledge it is likely that specimens may appear to differ to an extent needing a new genus, as happened with *Meggittina* and *Rajotaenia*. Further examination, coupled with the discovery of new forms partially filling the gap between genera, may lead to the kind of reassessment by which Wolfgang assigned *Meggittina* and his new species *aegyptica* to the genus *Catenotaenia*, and ultimately a situation is reached when a large variety of species are all ascribed to one genus. So long as these species present a continuous graded series of characters, even though the two ends of the series are worms of considerably different appearance, it is logical to retain the single generic name for all. But when, as in the opinion of the present author, the *Catenotaenia* series becomes readily divisible into two groups distinct in a number of separate characters, the situation allows or demands division into two genera. It is for this reason that *Skrjabinotaenia* has been adopted as the proper generic name for the new species *cricetomydis*.

With the removal of *Skrjabinotaenia* from *Catenotaenia* redefinition of these genera is desirable ; for *Catenotaenia* the diagnosis given by Wolfgang required only slight modification as follows.

Diagnosis of the genus **Catenotaenia**

The scolex is unarmed, with or without an apical sucker. Strobila generally long, with the gravid proglottides longer than their width. Testes numerous, arranged in one or two fields, the latter generally joined posterior to the ovary. Ovary dendritic, vitelline gland dendritic or compact, lying at the poral side of the ovary. The genitalia open together at the genital pore, these pores alternating irregularly, each lying in the anterior half of the lateral margin of its proglottis. The cirrus sac is generally straight and the vas deferens coiled only in the region near the cirrus sac. Seminal vesicles are absent. Vagina thick or thin walled, long or short, ending in a large receptaculum seminis. The uterus has a median stem occupying most of the length of the gravid proglottis, with lateral branches generally more than ten in number on each side, the number being a specific character. Eggs may or may not be embryonated. The excretory vessels may be simple or anastomosing.

Distribution :	Africa, Eurasia, North America.
Hosts and Location :	Intestine of myomorph and sciuromorph rodents.
Type :	*C. pusilla* (Goeze, 1782).

Diagnosis of the genus **Skrjabinotaenia**

The scolex is unarmed, generally with a vestigeal apical sucker. Strobila short or very short with the gravid proglottides generally wider than their length. Testes numerous, arranged in two antero-lateral fields and not extending behind the ovary. Ovary dendritic, vitelline gland of more compact lobes close to the receptaculum seminis on the poral side of the segment. The genitalia open together at the genital pores, which alternate with some regularity, each lying at the anterior limit of the lateral margin. The cirrus sac is generally curved, the vas deferens mainly straight but looped near the cirrus sac. Seminal vesicles are absent. Vagina distinct but thin walled, leading to a bipartite receptaculum seminis. The uterus has a short median stem, often with a pronounced anterior pocket, and from one to ten lateral branches on each side. The number of uterine branches is characteristic for each species, and some of them are commonly voluminous with occasional anastomoses. The eggs may or may not be embryonated. Longitudinal excretory vessels are sometimes numerous and may anastomose.

Distribution : Africa, Eurasia.

Hosts and Location : The intestine of myomorph rodents.

Type : *S. oranensis* (Joyeux and Foley, 1930).

Specimens of *S. cricetomydis* n.sp. are deposited in the Zoology Department of University College, Ibadan, Nigeria ; at the British Museum of Natural History ; and some are retained in the possession of the author at Southampton University.

Summary

1. *Skrjabinotaenia cricetomydis* n.sp. is described from 18 specimens taken from *Cricetomys gambianus* Waterhouse, near Ibadan, Nigeria.

2. Apolytic proglottides are described and also some details of development of the eggs.

3. Details of the anatomy are given and their significance in deciding the relationship with other genera and species is discussed.

4. The regrouping of a number of species into the genus *Skrjabinotaenia* is proposed.

5. The diagnostic features of the genera *Catenotaenia* and *Skrjabinotaenia* are restated.

Acknowledgments

It is a pleasure to record the many kindnesses received from Professor J. E. Webb and his staff at University College, Ibadan, during the course of this work. Thanks are also due to Dr. J. D. Thomas, University of Ghana, for the opportunity to discuss this and other helminthes collected in Nigeria ; to Mr. S. Prudhoe, British Museum (Nat. Hist.) and Professor J. J. C. Buckley, London School of Hygiene and Tropical Medicine, for their advice and specimens.

References

Akhumian, K. S., 1946.—"Systematics of the cestode genus *Catenotaenia* Janicki, 1904." Collected papers on Helminthology dedicated to K. I. Skryabin, 37–41.

Baer, J. G., 1951.—Ecology of Animal Parasites. Univ. Illinois Press. Urbana'

Baylis, H. A., 1927.—"The cestode genus *Catenotaenia*." *Ann. Mag. nat. Hist.*, **19,** 433–439. (W.L. 1050)

Clark, D. T., 1954.—"A new cyclophyllidean Cestode from the avocet (Nebraska)." *J. Parasit.*, **40,** 340–346. (W.L. 11428)

Dollfus, R. Ph., 1954.—"*Catenotaenia chabaudi* n.sp. de *Xerus* (*Atlantoxerus*) *getulus* Linné, 1758." *Miscellanea Helminthologica Maroccana X. Arch. Inst. Pasteur Maroc.*, **4,** 533–540.

Erhardova, B. and Ryšavy, B., 1955.—"Přispěvek k poznani cizopasných ćervu naišch myši a hrabošu." *Zool. a entomol. listy*, **4** (18). Quoted from Tenora, F.

Joyeux, C. E. and Baer, J. G., 1945.—"Morphologie evolution et position systematique de *Catenotaenia pusilla* (Goeze, 1782) cestode parasite de rongeurs." *Rev. suisse Zool.*, **52,** 13–51. (W.L. 19288)

——— and Foley, H., 1930.—"Les helminthes de *Meriones shawi* Rozet dans le nord de l'Algerie." *Bull. Soc. zool. Fr.*, **55,** 353–374. (W.L. 5401)

Lynsdale, J. A., 1953.—"On a remarkable new Cestode, *Meggittina baeri* gen. et sp. nov. (Anoplocephalinae) from rodents in Southern Rhodesia." *J. Helminth.*, **27,** 129–142. (W.L. 11224c)

Meggitt, F. J. and Subramanian, K., 1927.—"The tapeworms of rodents of the sub-family Murinae." *J. Burma Re. Soc.*, **17,** 190–237. (W.L. 11091)

Ortlepp, R. J., 1940.—"South African Helminths. Pt. VII. Miscellaneous helminths, chiefly Cestodes." *Onderstepoort J.* vet. Sci., **14,** p. 97. (W.L. 15681a)

Rosvear, D. R., 1950.—"Rodents of Nigeria." *Niger. Fld.*, **15,** 148–172. (W.L. 15128a)

Spasski, A. A., 1950.—"A new family of Cestodes, Catenotaeniidae n.fam. and a review of the system of Anoplocephalids". C. R. Acad. Sci. U.R.S.S., **75,** 597–599. (W.L. 7450)

———, 1951.—Principles of Cestodology. Ed. by K. I. Skryabin, vol. 1. Anoplocephalata. Moscow. Izdatelstvo Akademii Nauk. SSSR.

———, 1951.—"Metamerism in organisms and time factor." (In Russian). *C. R. Acad. Sci, U.R.S.S.*, **79,** 549–551 (*W.L. 7450*).

———, 1956.—"The relationship of the genera *Meggittina* Lynsdale, 1953 and *Skrjabinotaenia* Akhumian, 1946 to the family Catenotaeniidae Spasski, 1950." *Trud. gel'mint. Lab.*, **8,** 226–232. (W.L. 21817aa)

Tenora, F., 1959.—"Revision of the classification of Platyhelmia of the family Catenotaeniidae Spasski, 1950." *Zool. Zh.*, **38,** 1322–1334. (W.L. 23827)

Wertheim, G., 1954.—"A new Anoplocephalid cestode from the gerbil." *Parasitology*, **44,** 446–449. (W.L. 16035)

Wolfgang, R. W., 1956.—"*Catenotaenia aegyptica* sp. nov. from myomorph rodents, with additional notes on the genus." *Canad. J. Res.* (*Zool*)., **34,** 6–20. (W.L. 5898c)

Yamaguti, S., 1960.—Systema Helminthum, vol. 2, Cestodes of Vertebrates. Interscience.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 255–258.

On a New Cestode, *Paradilepis maleki* sp. nov. (Diplepididae), from a Sacred Ibis in the Sudan

By L. F. Khalil

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

Five complete gravid worms and several scolices and fragments of this species were collected by Dr. E. A. Malek from the intestine of a Sacred Ibis, *Treskiornis ethiopicus* (Latham) in Khartoum in the Sudan.

Paradilepis maleki sp. nov.

The largest worm measures 2·2 mm. in length with a maximum width of 0·2 mm. attained at the posterior end.

The scolex has a diameter of 0·26–0·3 mm. and is provided with four more or less spherical suckers measuring 0·082 mm. in diameter. The rostellum, diameter 0·1–0·11 mm., is armed with a double crown of hooks, twenty in number. The large hooks measure 89–96μ in length, and the smaller hooks measure 60–64μ in length. The short neck is immediately behind and narrower than the head. The segments become clearly defined a short distance behind the head; they are much wider than long.

There is a single set of genital organs in each segment. The genital pores are unilateral and open on the anterior third of the lateral margin of the segment. The male organs develop before the female organs. The testes are four in number, one central, two apporally, and one on the poral side. They gradually enlarge in size, and reach their maximum size when the ovary appears. They completely disappear shortly after the appearance of the ovary. The vas deferens is slightly coiled before entering the cirrus sac. The cirrus sac is comparatively large, pear shaped, with its widest part nearly touching the anterior border of the segments and measures 78–89μ by 35–38μ. The cirrus is armed with numerous spines.

The ovary is bilobed and lies in the centre of the segment. The vitelline gland is compact, more or less spherical, and lies posterior

to the ovary near the posterior border of the segment. The vagina is a straight thin-walled tube. The genital atrium is shallow and simple. The uterus is dumb-bell shaped when gravid.

Discussion

According to the published literature there are only three species of *Paradilepis* in which the length of the strobila is 10 mm. or less. These are : *P. scolecina* (Rudolphi, 1819) ; *P. urceus* (Wedl, 1855) ; and *P. minima* (Goss, 1914).

P. minima was considered by Mahon, 1955, as a synonym of *P. scolecina*. Clark (1957), disagreed with Mahon and showed that the material examined by Goss contained two species one of which is identified as *P. scolecina*, and the other as *P. minima*. Clark has redescribed *P. minima* and she has also re-established its validity.

The species under consideration cannot be assigned to any of the above three species, and consequently it is considered to be a new species for which the name *Paradilepis maleki* is proposed after Dr. E. A. Malek who entrusted the writer with the material.

P. maleki sp.nov. differs from *P. scolecina* in the definite external segmentation of the strobila, the smaller length of the hooks, and the disposition of the testes.

From *P. urceus* the new species differs in the shape of the worm, the length and number of hooks, the number of testes and the size of the cirrus sac.

P. minima has 28 hooks, and the hooks are larger in size than the 20 hooks of *P. maleki*.

Paradilepis maleki sp.nov.

Specific diagnosis : With the characters of the genus. Strobila reaches 2·2 mm. in length and 0·2 mm. in width. Scolex diameter 0·26–0·3 mm., with four suckers 0·082 mm. in diameter and a double crown of 20 hooks. Large hooks 89–96μ in length, small hooks 60–64μ in length. Four testes. Cirrus sac 78–89 $\times$ 35–38μ.

Host : *Treskiornis ethiopicus* (Latham)

Habitat : Intestine

Locality : Khartoum, Sudan

Type : Helminthological collection of the London School of Hygiene and Tropical Medicine

SUMMARY

A new cestode, *Paradilepis maleki* sp.nov. is described from the intestine of a Sacred Ibis in the Sudan, and differentiated from the other nearer species.

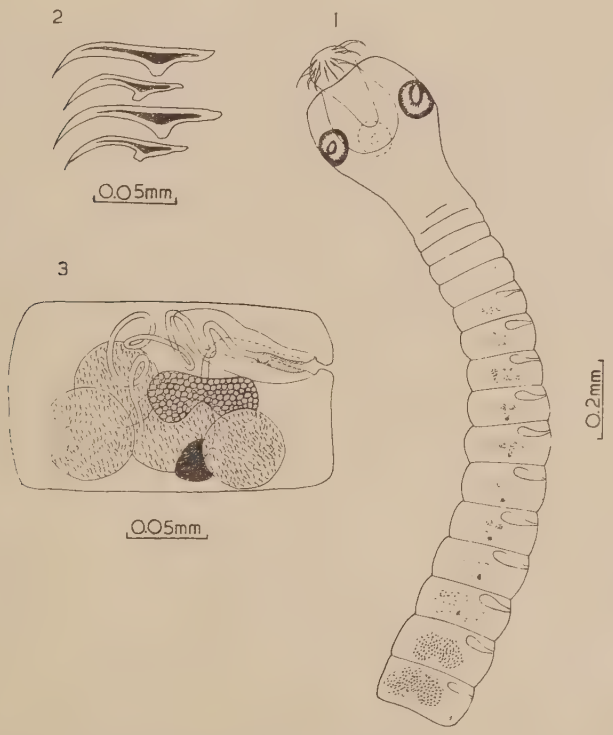


Fig. 1.—*Paradilepis maleki* n.sp., complete worm. Fig. 2.—Rostellar hooks. Fig. 3.—Mature segment.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Professor J. J. C. Buckley under whose guidance the work was done, to Dr. E. A. Malek for forwarding the material, and to Mr. S. Prudhoe of the

British Museum for lending material from the museum collection for comparison.

Finally I owe my thanks to the University of Khartoum for financial aid.

References

Clark, H. G., 1957.—"Cestodes from cormorants from South Australia." *Trans. roy. Soc. S. Aust.*, **80**, 124–134. (W.L. 21654)

Freeman, R. S., 1954.—"*Paradilepis rugovaginosus* n.sp. (Cestoda : Dilepididae) from the osprey, with notes on the genus *Oligorchis* Fuhrmann, 1906." *J. Parasit.*, **40**, 22–28. (W.L. 11428)

Hsü, H. F., 1935.—"Contributions a l'étude des cestodes de Chine." *Rev. suisse Zool.*, **42**, 447–570. (W.L. 19288)

Joyeux, C. and Baer, J. G., 1950.—"The status of the cestode genus *Meggitiella* Lopez-Neyra, 1942." *Proc. helm. Soc. Wash.*, **17**, 91–95. (W.L. 16747a)

Mahon, J., 1955.—"Contributions to the genus *Paradilepis* Hsü, 1935." *Parasitology*, **45**, 63–78. (W.L. 16035)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 259–262.

On a New Trematode, *Orientocreadium lazeri* sp. nov., from a Freshwater Fish, *Clarias lazera*, in the Sudan

By L. F. Khalil

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

This new species is a rare parasite of the freshwater fish *Clarias lazera* Cuvier and Valenciennes. Twenty-four fishes from the White Nile and the Blue Nile from Khartoum area in the Sudan were examined from October, 1957 to May, 1958. Only five fishes, all from the White Nile, were found to harbour this new species and each fish contained not more than two worms.

Orientocreadium lazeri sp. nov.

Body small elongate, cylindrical, measuring 1·5–2·3 mm. in length, with a maximum width of 0·37–0·56 mm. attained posterior to the ovary. Cuticle armed with fine spines which become sparse posteriorly and completely disappear posterior to the posterior testis. Both suckers are well developed and nearly equal in size. The oral sucker is rounded, subterminal, measuring 0·15–0·2 mm. in diameter while the acetabulum measures 0·16–0·22 mm. in diameter and lies in the anterior part of the middle third of the body. The mouth is separated from the pharynx by a short pre-pharynx measuring 0·03–0·05 mm. in length. Pharynx, 0·09–0·13 × 0·12–0·16 mm. is followed by a short oesophagus measuring 0·03–0·05 mm. in length. The caeca reach the posterior end of the body and are moderately wide.

The globular or subglobular equal testes are situated tandem, close to the dorsal wall in the anterior part of the posterior half of the body and measure 0·15 – 0·21 × 0·14–0·19 mm. The two testes are separated by a number of eggs. The cirrus sac measures 0·16–0·24 × 0·06–0·09 mm. and lies dorsal to the acetabulum and to one side of it, extending backwards slightly posterior to the acetabulum.

It encloses a well developed oval internal seminal vesicle measuring 0·09 × 0·06 mm., a pars prostatica and a cirrus which opens in the genital atrium which in turn opens to the outside just in front of the acetabulum. The external seminal vesicle is roughly spherical measuring 0·09–0·14 mm. in diameter and lies posterior to the cirrus sac.

The ovary is rounded or slightly oval measuring 0·12–0·15 mm. in diameter and lies at about the middle of the body, behind but not so far from the acetabulum. Laurer's canal is present and opens on the dorsal side. The uterus extends backwards near to the caudal end of the body. The irregularly shaped vitelline follicles extend on either side of the body from the ovarian level to the posterior end of the body where follicles from the two sides unite. A small vitelline reservoir is present between the anterior testis and the ovary.

The eggs are oval measuring 28·8–33·6 × 18–21 μ.

The excretory pore lies at the posterior end of the body and the excretory vesicle is sac-like.

Discussion

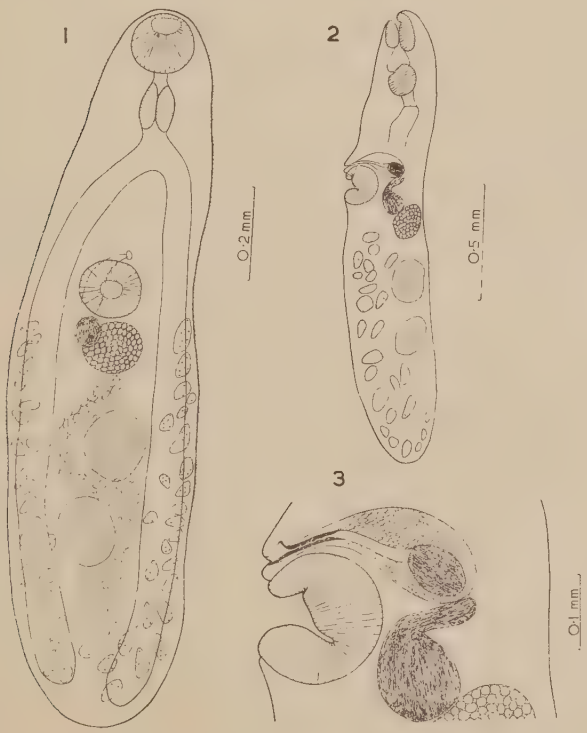
The genus *Orientocreadium* was erected by Tubangui (1931) to receive *O. batrachoides* collected from the freshwater fish *Clarias batrachus* from Philippine. He placed the genus in the family Allocreadiidae Stossich, 1904, and the subfamily Allocreadiinae Looss, 1902. Pande (1934) reported *O. indicum*, and Yamaguti (1934) reported *O. pseudobagria*, and both authors proposed to place the genus in the subfamily Plesiocreadiinae Winfield, 1929. Yamaguti (1958) erected a new subfamily Orientocreadiinae for this genus together with the genus *Macrotrema* Gupta, 1951.

Five genera were synonymised with the genus *Orientocreadium*. These are : *Ganada* Chatterji, 1933 ; *Neoganada* Dayal, 1938 ; *Nigamia* Dayal, 1938 ; *Ganadotrema* Dayal, 1949 ; *Paratormopsolus* Dubinina and Bychovsky, 1954.

The genus now contains twelve species, all reported from freshwater fishes from India, Japan, the Philippines and Russia. None of the species have so far been reported from Africa.

As it was not possible to assign the species under consideration to any of the existing twelve species of the genus *Orientocreadium*, it is considered to be a new species for which the name *O. lazeri* is proposed.

O. lazeri sp.nov. resembles to some extent *O. batrachoides* Tubangui, 1934, but it differs from it in the shape and size of the external seminal vesicle, the presence of a well developed internal seminal vesicle, the equal testes, and the host and geographical distribution.



Orientocreadium lazeri sp.nov.

Fig. 1.—Ventral view. Fig. 2.—Sagittal section. Fig. 3.—Region of cirrus sac in a sagittal section.

Orientocreadium lazeri sp. nov.

Specific diagnosis : With the characters of the genus *Orientocreadium* Tubangui, 1931. Body 1·5–2·3 mm. Oral sucker 0·15–0·2 mm. and acetabulum 0·16–0·22 mm. in diameter. Pharynx

0·09–0·13 × 0·12–0·16 mm., pre-pharynx and oesophagus very short. Testes equal 0·15–0·21 × 0·14–0·19 mm. with smooth margin. Cirrus sac 0·16–0·24 × 0·06–0·09 mm. External and internal seminal vesicle present. Ovary 0·12–0·15 mm. in diameter. Vitellaria extending from ovarian level to the posterior end of the body. Eggs 28·8–33·6 × 18–21 μ.

Host : *Clarias lazera* Cuvier and Valenciennes.

Habitat : Intestine.

Locality : White Nile, the Sudan.

Type : Helminthological collection, London School of Hygiene and Tropical Medicine.

References

Chatterji, R. C., 1933.—"On the trematode parasites of a Rangoon siluroid fish *Clarias batrachus* (Linnaeus, 1785)." *Bull. Acad. Sci. Unit. Prov.*, **3**, 33–40. (W.L. 3691c)

Dayal, J., 1938.—"Studies on the trematode parasites of fishes. A new trematode *Neoganada barabankiae* (nov.gen. nov.sp.) from *Clarias batrachus*." *Proc. Indian Acad. Sci.*, **7**, 132–137. (W.L. 16753a)

————, 1938.—"Studies on the trematode parasites of fishes. A new trematode *Nigamia hyderabadi* n.gen. n.sp. from the intestine of a freshwater fish, *Ophicephalus punctatus*." *Proc. nat. Acad. Sci. India*, **8**, 53–58. (W.L. 16820a)

————, 1949.—"Trematode parasites of Indian fishes. Part II." *Ind. J. Helminth.*, **1**, 93–120. (W.L. 9939g)

Gupta, S. P., 1951.—"Trematode parasites of Indian fishes. Three new trematodes of the subfamily *Leptophallinae* Dayal, 1938, from freshwater fishes of U.P." *Ibid.*, **3**, 29–40.

Pande, B. P., 1934.—"On a new trematode from an Indian freshwater fish." *Proc. Indian Acad. Sci.*, **4**, 107–112. (W.L. 16753a)

Tubangui, M. A., 1931.—"Trematode Parasites of Philippine vertebrates, III : Flukes from Fish and Reptiles." *Philipp. J. Sci.*, **44**, 417–424. (W.L. 16189)

Yamaguti, S., 1934.—"Studies on the Helminth Fauna of Japan, part 2. Trematodes of Fishes, I." *Jap. J. Zool.*, **5**, 249–537. (W.L. 10881w)

————, 1958.—Systema Helminthum—Digenetic Trematodes. New York.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 263–268.

On a New Nematode, *Raphidascaroides bishaii* sp. nov., from a Freshwater Fish, *Gymnarchus niloticus*, in the Sudan

By L. F. Khalil

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

Several females of this new species were collected by the writer from the stomach of the Nile fish, *Gymnarchus niloticus* Cuvier from Khartoum in the Sudan, on June, 1958. Three males and several females were collected from the same host and locality, by Dr. Hilmy Bishai of the Zoology Department, University of Khartoum on March, 1960 and forwarded to the writer.

Raphidascaroides bishaii sp. nov.

The males have a cylindrical body tapering gradually anteriorly, and rolled ventrally posteriorly. They measure 35–36 mm. in length and 0·75–0·93 mm. in maximum thickness. The cuticle is very finely striated. The head which measures 0·18 mm. in diameter at its base carries three lips, one dorsal, and two subventral, measuring 0·14 mm. in height. The lips are unequal, without dentigerous ridges but with lateral cuticular expansions and are lobed on their inner surface. The dorsal lip, which is larger in size compared to the subventral lips is provided with two double papillae, the more dorsal component of which is larger than the outer (see Figs. 1, 2, 3), and its pulp is in the form of a wide spoon carrying two wing-like processes internally. The two subventral lips carry only one double papilla each, of which the more dorsal part is the larger and their pulp also carries two bluntly-rounded processes internally (see Fig. 3). Interlabia are present measuring 0·054–0·064 mm. long. Lateral grooves are found at the base of the head between the lips and interlabia. The neck is slightly broader than the head and the cervical papillae could not be seen. The nerve ring lies at 0·53–0·57 mm. from the anterior end and the excretory pore lies at the same level or slightly posterior. The oesophagus, 2·25–2·85 mm. long, leads to a ventriculus 0·15–0·16 × 0·19–0·21 mm. and is produced backwards into an appendix 1·2–1·3 mm. long (see Fig. 6).

The tail is conical, 0·19–0·23 mm. long with its tip covered with rudimentary spines. The testes are filiform and much coiled. The spicules are slightly subequal, alate and pointed measuring 0·97–1.20 mm. in length. There are 22–24 pairs of preanal papillae and 2–3 pairs of very small postanal papillae. Adanal papillae are lacking. The preanal papillae become closer and smaller in size the nearer they are to the anus.

The females measure 50–65 mm. in length, with a maximum width of 1·3–1·65 mm. The head is 0·22–0·32 mm. in diameter at the base, with the lips 0·16 mm. high and the interlabia 0·06 -0·08 mm. long. The nerve ring lies at 0·60–0·83 mm. from the anterior end and the excretory pore lies at the same level or slightly posterior to it. The oesophagus is 3·0–3·5 mm. long ; the ventriculus 0·16–0·18 × 0·22–0·23 mm.; and the ventricular appendix 1·1–1·8 mm. long. The conical tail is 0·52–0·60 mm. long with its tip covered by minute spines. The vulva lies 14–18 mm. from the head dividing the body length in proportions of 1 : 2·5 approximately. The eggs are spherical measuring 0·047–0·05 mm. in diameter.

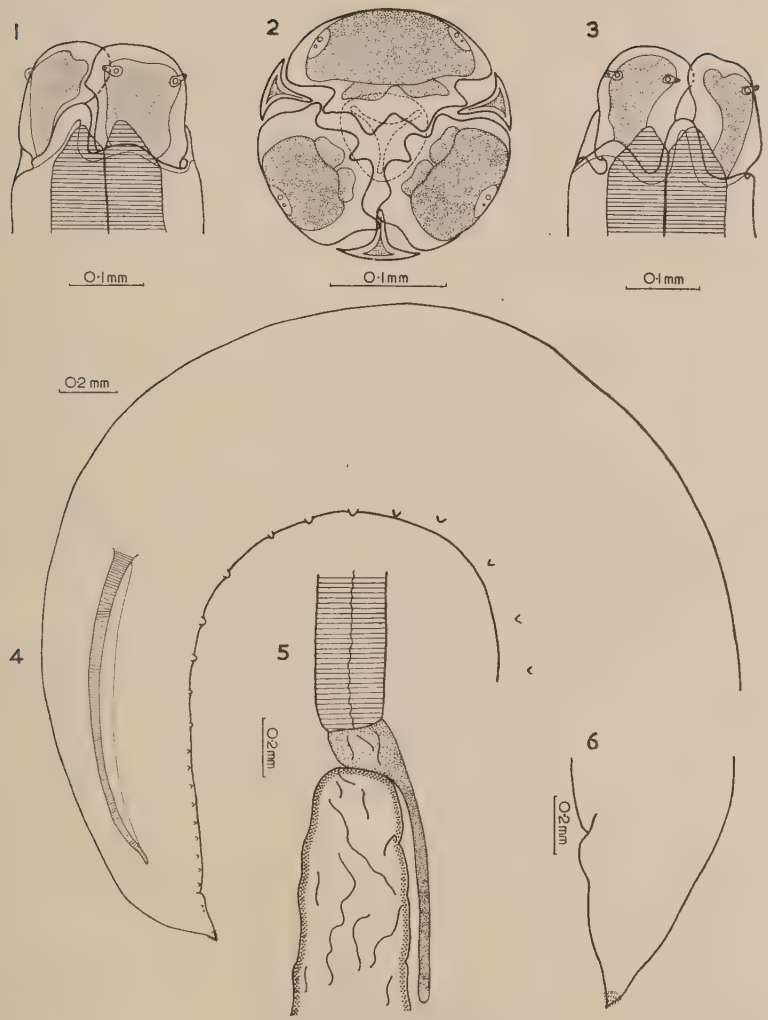
Discussion

Yamaguti (1941) erected the genus *Raphidascaroides* for worms belonging to the subfamily Anisakinae Railliet and Henry, 1912 which possess a ventricular appendix and interlabia. This genus is intermediate between *Raphidascaris* Railliet and Henry, 1915 which lacks the interlabia but possess a ventricular appendix and *Paranisakis* Baylis, 1923 with interlabia but without a ventricular appendix.

One species, *Raphidascaroides nipponensis* Yamaguti, 1941 and a subspecies *R. nipponensis lophii* Yamaguti, 1941 were attached to this genus at that time, both from marine fishes.

Thwaite (1927) described *Raphidascaris diodonis* from the intestine of the marine fish *Diodon hystrix* from South India. Mosgovi (1951) erected the genus *Ryjikovascaris* for this species. Olsen (1952) transferred *Raphidascaris diodonis* to *Raphidascaroides* while Hartwich (1957) synonymised the genus *Ryjikovascaris* with the genus *Raphidascaroides*. I believe that this is justified.

Hartwich (1954) erected the subfamily Raphidascaridinae which included five genera ; *Raphidascaris* Railliet and Henry, 1915 ; *Heterotyphlum* Spaul, 1927 ; *Paranisakis* Baylis, 1923 ; *Raphidascaroides* Yamaguti, 1941 and *Thynnascaris* Dollfus, 1933.



Raphidascaroides bishaii n.sp.

Fig. 1.—Dorsal view of head. Fig. 2.—*En face* view. Fig. 3.—Ventral view of head. Fig. 4.—Posterior end of male. Fig. 5.—Oesophagus, ventriculus, ventricular appendix and intestine. Fig. 6.—Posterior end of female.

As far as I can find out from the published literature only the above two species and subspecies are currently referred to the genus *Raphidascaroides*, and all are from marine fishes. I assume that some other species belonging to this genus may have been misplaced in the genus *Paranisakis* as the difference between the two genera lies in the presence or absence of the ventricular appendix, a character which could be easily overlooked unless the worms were dissected, or as Olsen (1952) stated, that the descriptions of several species of *Raphidascaris* do not make clear whether interlabia are present or absent, and an examination of type specimens of these species may show that some of them should be transferred to *Raphidascaroides*.

The above described species is considered here to be new and the name *Raphidascaroides bishaii* is proposed for it after Dr. Hilmy Bishai who kindly entrusted his material to me for study.

R. bishaii sp.nov. differs from *R. nipponensis* Yamaguti, 1941, and *R. nipponensis lophii* Yamaguti, 1941 in the measurements of the different organs, the absence of dentigerous ridges, the subequal spicules, the number of postanal papillae, the position of the vulva, the host being a freshwater fish and in its geographical distribution.

From *R. diodonis* (Thwaite, 1927) *R. bishaii* sp.nov. differs in the measurements of the different organs, the size of the spicules, the absence of a median preanal papilla, the number of preanal and postanal papillae, the position of the vulva and the size of the eggs ; it also differs in the host and the geographical distribution.

I believe the above differences are sufficient to justify the erection of a new species.

Raphidascaroides bishaii sp. nov.

Specific diagnosis : With the characters of the genus. Males 35–36 mm. long and 0·75–0·93 mm. wide. Spicules subequal, alate, 0·97–1·2 mm. long. 22–24 pairs of preanal papillae and 2–3 pairs of small postanal papilla. Females 50–65 mm. long and 1·3–1·65 mm. wide. Vulva lies at 14–18 mm. from the anterior end. Eggs 0·047–0·05 mm. in diameter. Parasites of freshwater fishes.

Host : *Gymnarchus niloticus* Cuvier.

Habitat : Stomach.

Locality : Khartoum, Sudan.

Type : Department of Parasitology, London School of Hygiene and Tropical Medicine.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Professor J. J. C. Buckley, under whose guidance the work was done, to Dr. Hilmy Bishai for the material and to Dr. W. G. Inglis for his interest in the study. I owe my thanks to the University of Khartoum for financial help.

REFERENCES

HARTWICH, G., 1954.—"Die Vorderdarmstrukturen, das Exkretionssystem sowie der Kopfbau der Ascariden und ihre taxonomische Bedeutung." *Wiss. Z. Univ. Halle*, Math.-Nat. Reihe, **3**, 1171–1212.

———, 1957.—"Zur Systematik der Nematoden-Superfamilie Ascaridiodea." *Zool. Jber.*, **85**, 211–252. (W.L. 23835)

OLSEN, L. S., 1952.—"Some nematode parasite in marine fishes." *Publ. Inst. Mar. Sci. Univ. Tex.*, **11**, 173–215. (W.L. 17368F)

SKRJABIN, K. I., SHIKHOBALOVA, N. P. and MOSGOVOI, A. A., 1951.—"Descriptive catalogue of Parasitic Nematodes. Volume 2. Oxyurata and Ascaridata." Moscow "Russian Text"

THWAITE, J. W., 1927.—"On a collection of Nematodes from Ceylon." *Ann. trop. Med. Parasit.*, **21**, 225–244. (W.L. 1063)

YAMAGUTI, S., 1941.—"Studies on the Helminth Fauna of Japan. Part 33. Nematodes of fishes." *Jap. J. Zool.*, **9**, 343–396. (W.L. 10881w)

ADDENDUM

After this paper was sent for publication, Yamaguti (1961) described a new species from a marine fish, *Chilomycterus affinis* in Japan which he named *Raphidascaroides chilomycteri*.

R. bishaii sp.nov. differs from this species not only in the body length but also in the measurements of the different organs, size of the spicules, number of preanal and postanal papillae and in the size of the eggs.

Reference

Yamaguti, S., 1961.—"Studies on the Helminth Fauna of Japan. Part 57. Nematodes of Fishes, III." *J. Helminth., R. T. Leiper Supplement*, 217–228. (W.L. 11224c)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 269–274.

On the Capture and Destruction of Miracidia by *Chaetogaster limnaei* (Oligochaeta)

By L. F. Khalil

From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

In an attempt to demonstrate experimentally the life cycle of *Fasciola gigantica*, the snails *Lymnaea natalensis* were exposed to newly hatched miracidia of this trematode.

Eggs of *F. gigantica* were supplied by the Uganda Veterinary Department through Mr. T. Coyle and snails were provided by Dr. P. L. LeRoux ; the snails originally came from three localities, the Sudan, the Congo and Rhodesia, and had been colonised in the aquaria of the London School of Hygiene and Tropical Medicine for some time.

Well-grown snails were exposed individually to 5–10 newly hatched miracidia in 3 in. × 1 in. tubes. The snails and the miracidia were kept in about 1 in. of water for 12–15 hours after which time they were removed to separate aquaria marked with the date of exposure and the country of origin. When new generations of snails were available, specimens of different ages were exposed to miracidia ; ultimately nearly 500 snails of all ages had been exposed to miracidia in different batches.

After ten days of exposure one or two snails of every batch were examined for developmental stages of the trematode but the expected infection was not revealed in any of them. Examination continued at 15, 21 days and then at irregular intervals for ten weeks after exposure, but still no developmental stages were recovered. It became evident that for some unknown reason these batches of snails did not contract the infection.

A few snails were then exposed as before, but to a heavier dose of miracidia (over a hundred) and the snails used were only half grown ; as before, the snails were exposed individually. When

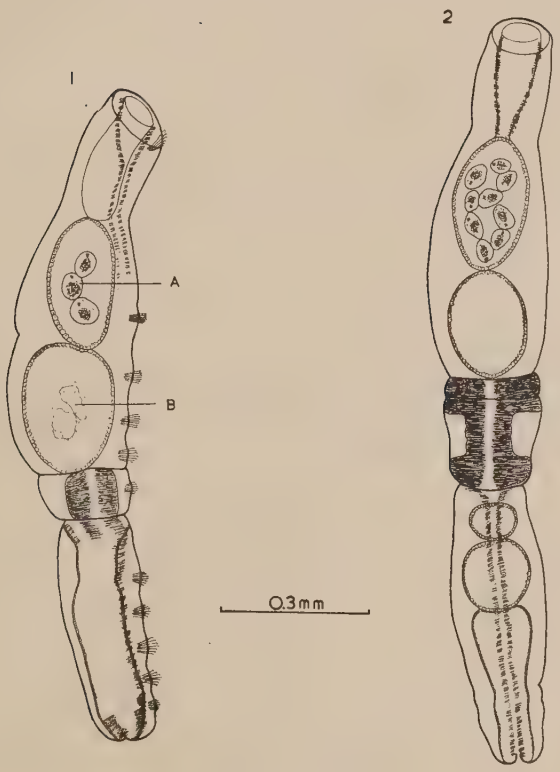
examined later some of them were found infected and developmental stages were recovered, and by the end of 24–32 days of exposure some well developed rediae were found.

During the course of this work it was found that nearly all the snails used were infested externally with the oligochaet worm, *Chaetogaster limnaei* K. von Baer, 1872. Each snail had at least 7–10 worms and as many as 50 were recovered from a single snail. It was observed that the younger the snails the fewer the number of worms attached, on the other hand very old snails also had few of these worms. The highest infestation rate was found among half-grown snails.

Chaetogaster limnaei is not a parasite of the snails, but a commensal, attached to the soft tissue underneath the shell by its setae or bristles posteriorly, while its anterior part is free. The white body is divided into somites which carry bundles of setae ventrally and is capable of considerable extension. Although they remain inside the shell of the snail they occasionally extend the anterior part of their body outside the shell, and sometimes they even creep out and leave the snail completely. They then move very slowly in the water and it seems that they find their way to their snail "host" only by chance.

When an infested snail was placed in a small dish with a little water and a few miracidia were added to the water, the activity of the worms was seen to increase after a few moments. The body is stretched considerably and the worms endeavour to catch and swallow the swimming miracidia, but owing to the fast movement of the miracidia, their efforts were mostly unsuccessful. However, most of the miracidia that went inside the snail shell, where the worms were situated, were never seen coming out, and when any miracidium ceased swimming and attached itself either to the head, tentacles or part of the foot of the snail, it was quickly ingested by a *Chaetogaster*. When after half an hour the snail was crushed and the worms were freed from the snail tissue, the majority of them had a variable number of miracidia in their gut, up to 15. By that time the miracidia were not yet digested by the worms and their outline and eyespots could be very clearly seen in the gut.

It seems that not all species of snails are susceptible to infestation with *Chaetogaster limnaei*, and in the aquaria of the London School of Hygiene and Tropical Medicine where a large number of various snails are maintained, only *Lymnaea natalensis*, *L. acuminata*, *Bulinus truncatus*, *B. tropicus* and *Biomphalaria glabratus* were found infested.



Chaetogaster limnaei

Fig. 1.—Lateral view of worm.
A.—miracidia newly ingested. B.—miracidia partly digested.

Fig. 2.—Dorsal view.

Discussion

It is very difficult to explain the reason for the failure in establishing infection in the snails exposed to 5–10 miracidia each in the first experiment. The snail used was *Lymnaea natalensis*, the normal natural host in Africa of *F. gigantica* and the eggs were also from Africa. According to published literature successful infection was achieved by a single miracidium and some workers concluded that 10 miracidia was the maximum number that could be used without damaging the snail to such an extent that it would die during the development of the parasite. The success achieved later when snails were exposed to a heavier dose of miracidia suggests that the number of miracidia used in the initial experiments was not enough.

As the snails were later found to be infested with *Chaetogaster limnaei*, it seems possible that these worms were capable of ingesting the ten miracidia to which the snail was exposed, and in this way prevented their penetration. The worms were capable of taking a large number of miracidia and up to 15 were found in their gut. Probably very few miracidia were able to penetrate the snail even when a large dose was given.

For slow moving animals such as *Chaetogaster*, the mantle cavity of the snail must provide excellent protection against enemies. But in nature the snails are not as heavily infested with these worms as they are in aquaria in the laboratory. Backlund (1949) found no more than 10 worms per snail on *Lymnaea stagnalis* and *L. ovata* in nature. This could be explained on the assumption that these worms find their "hosts" by chance, and their numbers are kept down by natural enemies which ingest them when they are outside the mantle cavity of the snails. It is apparent that the infestation rate has built up rapidly during the course of this work in the aquaria for as many as fifty worms have been found on a single snail.

The food supply in a closed cavity must be very limited. There are records of cercariae as well as miracidia having been found in the gut of the *Chaetogaster*. Experimentally they were fed on paramoecia, and probably other micro-organisms will serve as food for them. Under natural conditions the food supply is probably never unlimited as it is in the laboratory. But it seems that at certain times of the year, particularly the warmer seasons, there

is a better food supply as cercariae will be emerging in greater numbers. Backlund (1949) found that a number of only slightly digested cercariae were pressed out through the anus of the worms when he was examining specimens whose gut was quite full of cercariae in the warm months. Some others assume that these worms eat their snail "host" when food is not available and so call them parasites; others define them as commensals as they do not harm the snails, while still others consider that the term "commensals" is strictly speaking not correct as they are predaceous animals.

References to these worms in the helminthological literature are scarce but not completely neglected. That they ingest trematode larvae is beyond doubt. Wagin (1931) found cercariae in the gut of the *Chaetogaster* and Wesenberg-Lund (1934) confirmed his findings. Backlund (1949) also found cercaria in the gut of these worms. Ruiz (1951) observed *Chaetogaster* frequently ingesting cercaria of *Schistosoma mansoni*, while Coelho (1957) observed miracidia of *S. mansoni* in the gut. Wallace (1941) reported that these worms serve as second intermediate hosts for some trematodes and when cercariae of *Triganodistomum mutabile* were ingested by *Chaetogaster limnaei* they penetrated the gut wall and encysted in the body cavity. When the worms with their metacercariae were fed to fishes the adult trematodes were later recovered.

There seems to be an inverse relation between the degree of infection of trematodes and the number of *Chaetogaster* infesting the molluscan host. However, too little is known to enable a far reaching conclusion to be drawn. There are different fields in this problem awaiting investigation. Is there a connection between the time of the year when food supply is abundant and the sexual maturity of the worms? What snails are susceptible to infestation with the worm? What type of cercariae are capable of penetrating the gut wall and what cercariae are easily digested? An increase in our knowledge of these worms will enable us to determine the part they play in nature in limiting the distribution of trematodes.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Professor J. J. C. Buckley under whose guidance the work was done, to Dr. P. L. LeRoux and Mr. T. J. Coyle for the material and interest in the work.

References

Backlund, H. O., 1949.—"En kommensal som äter sitt värddjurs parasiter." *Fauna o. flora*, **44**, 38–41. (W.L. 8336)

Coelho, M. V., 1957.—"Aspectos do desenvolvimento das formas larvias de *Schistosoma mansoni* em *Australorbis nigricans*." *Rev. bras. Biol.*, **17**, 325–337. (W.L. 18800d)

Ruiz, J. M., 1951.—"Nota sôbre a cercariofagia de um Oligochaeta do genero *Chaetogaster* v. Baer, 1827." *An. Fac. Farm. Odontol. Univ. São Paulo*, **9**, 51–56.

Wagin, W. L., 1931.—"*Chaetogaster limnaei* K. v. Baer als Cercarienvertilger." *Zool. Anz.*, **95**, 55–59. (W.L. 23833)

Wallace, H. E., 1941.—"Life history and embryology of *Triganodistomum mutabile* (Cort) (Lissorchiidae, Trematoda)." *Trans. Amer. micr. Soc.*, **60**, 309–326. (W.L. 21400)

Wesenberg-Lund, C., 1934.—"Contributions to the development of the Trematoda Digenea. II. The biology of the freshwater cercariae in Danish freshwaters." *K. danske vidensk. Selsk.*, **5**, 1–223. (W.L. 11861)

Willcox, M. A., 1901.—"A parasitic or commensal oligochaete in New England." *Am. Nat.*, **35**, 905–909. (W.L. 651)

Chaetogaster limnaei

(A). Lateral view of complete worm. X 108. (Same specimen as in Text Fig. 1.)
(B). Dorsal view of incomplete worm. X 125.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 275–284.

Studies on Larval Trematodes Infecting Freshwater Snails in London (U.K.) and Some Adjoining Areas.

Part IV. Schistosomatid Cercariae*

By D. Khan

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

During the last two years an investigation into the occurrence of freshwater larval trematodes was carried out in London and some parts of Kent, Essex and Middlesex. Four species belonging to the "Elvae" group of Miller's (1926) "Apharyngeal brevifurcate distome" division of Furcocercous cercariae were found. *C. pseudocellata* Szidat and Wigand, 1934 was found to be infecting *Limnaea stagnalis* (Linn.) in Lake Meadows, near Billericay (Essex) and *L. pereger* in Bushy Park and *C. bilharziellae polonicae* Szidat, 1929 was found parasitizing *Planorbis corneus* (Linn.) also in Lake Meadows. The infection rates were 10 and 5 per cent respectively. Both these cercariae have previously been recorded from other parts of Britain. The present paper deals with two more species which are new.

The infected snails were kept in the laboratory and the cercariae were studied both in the living and fixed conditions. For fixation 10% hot (70–80°C.) formal-saline was used. Of each species the measurements were taken from fifty naturally emerged cercariae.

1. *Cercaria kenilworthensis* n.sp.

About 10% of *Planorbis planorbis* (Linn.) collected from a small pond in Stoneyfields Park, Kenilworth Estate (Edgware) during the last half of September and the first half of October, 1959, were found to be discharging this cercariae. The molluscan fauna of this pond comprised, in addition, *L. stagnalis*, *L. pereger* (Müll.), *P. corneus* and *Hydrobia jenkinsi* Smith, but none of these species was ever found to be infected with this cercaria. This cercaria was never encountered in this pond during 1958, although the latter was under regular observation during that year.

* Part of a thesis approved by the University of London for award of the Ph.D. degree.

Sporocysts

A comparatively small number of long, thread-like sporocysts was found in the digestive gland of the host. The fairly transparent sporocysts are very delicate and so intricately intercoiled that it was impossible to separate them, without breaking. They are constricted at several places along their length. Each constriction is followed by a bulbous portion, which accommodates one or two developing cercariae. Some of these cercariae, apparently fully developed, continue lashing their tails briskly.

The largest portion of a sporocyst was 1100μ long, with a minimum breadth of 45μ and maximum breadth of 150μ.

Emergence and Behaviour

The cercariae are discharged during the day in comparatively small numbers. They exhibit a positive phototropism but do not swim directly to the lighted side of the container. Very often they are found hanging in the middle of the container. During this resting period the cercaria gradually sinks, keeping its furca closed and uppermost, while the tail and the body is in a straight vertical line.

If undisturbed for some time after their emergence, practically all of the cercariae are found attached to the side of the container nearest to the light. They attach with their ventral suckers, and keep the body and the tail in a straight vertical line, very close to the side of the container. The furcae in attached cercariae are kept closed but, contrary to the freely hanging cercaria, are lowermost.

The cercariae also have a tendency to remain in the upper level of the water. The life of free swimming cercariae is up to seventy-two hours at room temperature.

Cercariae

The elongated contractile body has two prominent darkly pigmented eyes, each with a lens on the outer side. The anterior organ is divided into a large glandular and a small muscular portion. There is a well developed head gland in the glandular part of the anterior organ. A narrow straight oesophagus proceeds backwards to a short distance behind the eyespots, where it divides into two short caeca. The well developed ventral sucker is behind the middle of the body and is highly protrusible. Because of this protruded ventral sucker the cercaria always lies on its lateral side under the coverglass on a slide. Four sets of muscles from the ventral sucker are attached to the dorsal body wall.

There are five pairs of penetration gland cells. The anterior two, with coarsely granular cytoplasm, are circum-acetabular and the posterior three, with clear cytoplasm, are behind the ventral sucker. The penetration gland ducts are very prominent and open at the tip of the anterior organ. The openings of these ducts are highly reinforced and capped with prominent conical processes.

The excretory bladder is small and rectangular. The main excretory ducts are coiled at the level of the ventral sucker. These coils have ciliary patches in them. The anterior collecting duct receives capillaries from three flame cells and the posterior from four, three in the post-acetabular region of the body and one of the proximal part of the tail. The caudal excretory duct traverses the whole length of the furca and opens on its tip into a cup-like structure which is demarcated off the main part of the furca.

The genital rudiment is represented by a small mass of deeply staining cells, lying just behind the ventral sucker.

The tail shaft is longer than the body and has uncrenulated margins, with strongly developed oblique muscles originating at the lateral margins and terminating at the centre. The furcae are narrow, blade-like, with a finfold extending the whole length on the dorsal and the ventral sides and continuous over the tip. They are not markedly constricted off the tail shaft.

The body, tail shaft and the furcae are uniformly spined.

MEASUREMENTS OF CERCARIAE

(All measurements in microns)

	Living		Fixed	
	Range	Average	Range	Average
Body length	433–550	469	300–336	321
breadth	110–120	115	83–93	90
Tail shaft length	583–700	653	533–600	561
breadth	66	66	53–63	57
Furca length	300–376	340	263–270	252
breadth at base	31–43	41	30–33	31
Anterior organ length	86–133	117	100–116	107
breadth	70–80	75	53–63	55
Ventral sucker diameter	40–56	50	30–36	33
Diameter of eye	11–13	12	10–13	12
Distance between eyes	—	—	18–22	20
Distance between eyes and anterior end of body	150–166	160	113–133	123
Distance between centre of ventral sucker and posterior end of body	—	—	100–133	108

Comparison with Related Species

The present species differs from *C. tuckerensis* Miller, 1927; *C. indicae XLVII* Sewell, 1922; *C. bilharziellae yokogawii* Oiso, 1927; *C. jaenschi* Johnston and Cleland, 1937; *C. elongata* Brackett, 1940; *C. gigantobilharzia sturniae* (Tanabe, 1948) Komiya and Ito, 1952; *C. duboisi* Fain, 1953 and *C. turicensis* Meyer and Dubois, 1954 in its flame cell formula. It differs from *C. schwetzi* Fain, 1953; *C. macrosoma* Brown, 1926 and *C. stagnicolae* Talbot, 1936 in its number of penetration gland cells. It differs from *C. variglandis* Miller and Northup, 1926 and *C. columbiensis* Edwards and Jansch, 1955 both in number of flame cells and penetration gland cells.

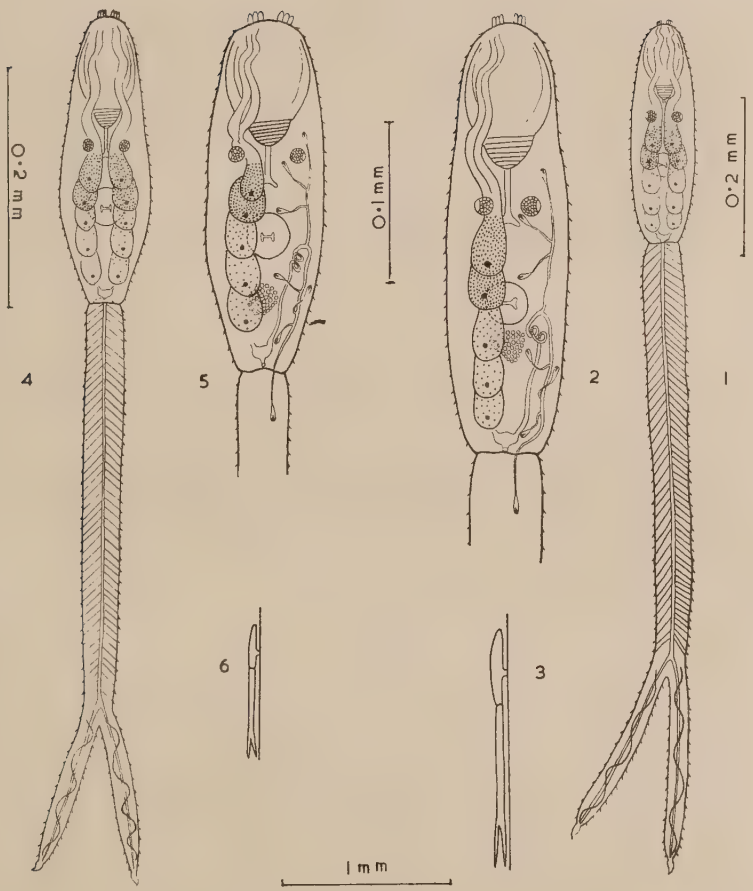
In having five pairs of penetration gland cells and a flame cell formula of 2×3—3—(1) 14, the present species resembles *C. ocellata* La Val., 1855; *C. elvae* Miller, 1923; *C. b. polonicae* Szidat, 1929; *C. echinomorpha* Brown, 1931 *C. parocellata* Johnston and Simpson, 1939; *C. pseudocellata* Szidat and Wigand, 1934; *C. trichobilharzia szidati* Neuhaus, 1952 (*C. parocellata* Szidat, 1942); *C. neocellata* Szidat, 1942; *C. oregonensis* Macfarlane and Macy, 1946; *C. dermolestes* McLeod, 1940; *C. longicauda* Macfarlane, 1944; *C. t. adamsi* Edwards and Jansch, 1955 and a marine form *C. littorinallinae* Penner, 1950.

The present cercaria can be separated from all the species referred to above because of its larger size and the position of the body and tail during its attachment to the illuminated side of the container. *C. subocellata* (Porter, 1938) Meyer and Dubois, 1954 also has a similar flame cell formula and the same number of penetration gland cells, but differs in being smaller, in the position of the anterior two pairs of penetration gland cells and in its behaviour. The resting posture of *C. herini* Fain, 1955 is not known, but can be distinguished from the present species in having a much smaller tail shaft, in the possession of an additional pair of flame cells in older cercariae and in having long hair-like structures on the body.

It is concluded that the cercaria studied here is a new species for which the name *Cercaria kenilworthensis* is proposed.

2. *Cercaria edgwarensis* n.sp.

About 12% of *P. planorbis* collected from a small pond in Stoneyfields Park, (Edgware) during September and October, 1959, were found to be discharging this cercaria. As in the case of *C. kenilworthensis* (present study) this cercaria was never recovered from any other species of snail from this pond, nor was it found during 1958.



Cercaria kenilworthensis n.sp.

Fig. 1.—General structure of the body, tail and furca. Fig. 2.—Detailed morphology of the body. Fig. 3.—Position of the cercaria during attachment.

Cercaria edgwarensis n.sp.

Fig. 4.—General structure of the body, tail and furca. Fig. 5.—Detailed morphology of the body. Fig. 6.—Position of the cercaria during attachment.

Sporocyst

The digestive gland of the host was teeming with a large number of sporocysts. These are long, thread-like and very delicate, with the same structure as described for *C. kenilworthensis* and similarly inextricably intercoiled. The cercaria while still enclosed in the sporocyst lashes its tail vigorously, with the result that portions of the sporocyst appear to be very motile, whereas it is actually inactive.

The largest portion of the sporocyst measured 1800μ with a minimum thickness of 30μ and a maximum thickness of 130μ.

Emergence and Behaviour

The cercariae are emitted during the day in large numbers. They show a strong phototropism and swim directly towards the light. They attach to the lighted side of the containers in exactly the same manner as described for *C. kenilworthensis*. On reversing the sides of the container or stirring the water the cercariae detach themselves, swim to the lighted side of the container and attach again. These cercariae also exhibit a tendency to aggregate near the surface of the water.

Cercaria

The body is highly contractile and uniformly spined all over. There are two prominent darkly pigmented eyespots, with lenses on the outer sides. The anterior organ is well developed with an anterior glandular and a posterior muscular portion. A well developed head gland is present. The gut is narrow and thin walled and terminates in two short rami slightly behind the eyespots. There is no trace of a pharynx.

The ventral sucker which is behind the middle of the body, unlike *C. kenilworthensis* and several other members of the group, is only very slightly protrusible and it is always possible to have the cercaria lying dorso-ventrally under the cover-glass on a slide.

There are five pairs of penetration gland cells in naturally emerged cercariae. The first on each side is the smallest and is partially enveloped by the second. First two of these glands have coarsely granular cyptoplasm while the posterior three have clear cytoplasm. The penetration gland ducts have a slightly convoluted course and

open at the anterior end ; their openings are very heavily reinforced, forming prominent cap-like projections at the anterior end.

The excretory system is similar to that described for *C. kenilworthensis*.

The genital rudiment is represented by a small mass of deeply staining cells lying between the ventral sucker and the posterior end of the body.

The tail shaft is longer than the body, with uncrenulated margins. Several oblique muscles originate at the lateral margins and terminate near the centre of the tail. The furcae are shorter than the body and are provided with a narrow finfold extending on its entire dorsal and ventral side and continuous over the tip.

MEASUREMENTS OF CERCARIAE

(All measurements in microns)

	Living		Fixed	
	Range	Average	Range	Average
Body length	250–290	270	240–276	261
Body breadth	100–110	109	83–100	90
Tail shaft length	300–360	342	316–366	342
Tail shaft breadth	30–40	38	30–40	36
Furca length	133–196	165	100–156	131
Furca breadth at base	16–20	17	13–20	16
Anterior organ	86–93x 60–66	90x 64	76–90x 46–53	85x 50
Ventral sucker	30–33x 30–33	31x 32	23–30x 30–36	26x 32
Distance between eyes	33–40	37	20–26	23
Distance between eyes and anterior end	106–113	108	90–106	99
Diameter of eye	—	—	9–10	10
Distance between centre of ventral sucker and posterior end of body	—	—	70–90	88

COMPARISON WITH RELATED SPECIES

C. tuckerensis Miller, 1927 ; *C. Indicae XLVII* Sewell, 1922 ; *C. bilharziellae yokogawii* Oiso, 1927 ; *C. jaenschi* Johnston and Cleland, 1937 ; *C. elongata* Brackett, 1940, *C. g. sturniae* (Tanabe, 1948) Komiya and Ito, 1952, *C. duboisi* Fain, 1953 and *C. turicensis* Meyer and Dubois, 1954, all have a flame cell pattern different from 2(3 3 (1)) 14. *C. schwetzi* Fain, 1953 has been described with four pairs of penetration gland cells and *C. macrosoma* Brown, 1926 with only two pairs. The present species with five pairs of penetration

gland cells and a flame cell formula of 2(3 3 (1)) 14 can readily be separated from all the species mentioned above, and from *C. variglandis* Mill and Northup, 1926 and *C. columbiensis* Edwards and Jansch, 1955 which have a different number of flame cells and penetration gland cells.

C. ocellata La Val. 1855 ; *C. elvae* Miller, 1923 ; *C. pseudocellata* Szidat and Wigand, 1934 ; *C. t. szidati* Neuhaus, 1952 (*C. parocellata* Szidat, 1942) ; *C. longicauda* Macfarlane, 1944 and *C. oregonensis* Macfarlane and Macy, 1946, are all larger than the present cercaria and also differ in the position of the body and tail during attachment. The cercaria under study has a shorter body and tail stem and much shorter furcae than *C. parocellata* Johnston and Simpson, 1939 and *C. neocellata* Szidat, 1942 and differs also in the way of attachment. *C. t. adamsi* Edwards and Jansch, 1955 has a smaller body but larger furcae and a different posture during rest.

C. stagnicolae Talbot, 1936 ; *C. dermolestes* McLeod, 1940 and *C. elongata* Brackett, 1940 do not attach to the surface, but hang motionless near the lighted side of the container. *C. physallae* Talbot, 1936 on the contrary attaches itself near the bottom of the container with its pre-acetabular region of the body and tail projecting upwards. The present species attaches to the side of the container, keeping its body and tail parallel to the side on which it is attached.

The present species also differs from *C. bilharziellae polonicae* Szidat, 1929 in having a larger body and tail shaft and much larger furcae ; and from *C. gyrauli* Brackett, 1940 in having a larger body and tail shaft but shorter furcae. It also differs from the latter two species and a marine form *C. littorinallinae* Penner, 1950 in that it does not cling to the surface of the water.

C. echinomorpha Brown, 1931 is quite close to the present species in measurements, although the body and furcae are slightly shorter and the tail shaft slightly longer in the former. The present cercaria differs from *C. echinomorpha* in that the oesophagus does not reach the ventral sucker ; the penetration glands are differentiated into two groups ; it does not float passively on the surface or continue lashing its tail when attached. In its posture during attachment it agrees with *C. kenilworthensis* (present study) but is much smaller in size.

C. herini Fain, 1955 is much larger and has a different flame cell pattern in older cercaria and long hair-like structures which are absent in the present species. *C. subocellata* (Porter, 1938) differs in being much larger and also differs in its behaviour during its free swimming life.

It is therefore concluded that the present species is new and the name *C. edgwarensis* is proposed for it.

SUMMARY

Cercaria pseudocellata Szidat and Wigand, 1934 ; *C. bilharziellae polonicae* Szidat, 1929 ; *C. kenilworthensis* n.sp. and *C. edgwarensis* n.sp. are recorded from London. Descriptions of the two new species and a comparison with related forms is given.

ACKNOWLEDGMENTS

I am greatly indebted to Professor J. J. C. Buckley for his most valuable guidance and encouragement in carrying out this work.

REFERENCES

BRACKETT, S. 1940.—"Two new species of Schistosome cercariae from Wisconsin." *J. Parasit.*, **26,** 195–200. (W.L. 11428)

————, 1942.—"Five new species of avian Schistosomes from Wisconsin and Michigan, with life cycle of *Gigantobilharzia gyrauli* (Brackett, 1960)." *Ibid.*, **28,** 25–42.

BROWN, F. J., 1926.—"Some British freshwater larval Trematodes with a contribution to their life-histories." *Parasitology*, **18,** 21–34. (W.L. 16035)

————, 1931.—"Some freshwater larval Trematodes from Cheshire." *Ibid.*, **28,** 88–98.

CORT, W. W. and TALBOT, S. B., 1936.—"Studies of Schistosome dermatitis. III. Observations on the behaviour of the dermatitis-producing Schistosome cercariae." *Amer. J. Hyg.*, **23,** 385–396. (W.L. 600a)

EDWARDS, D. K. and JANSCH, E. M., 1955.—"Two new species of dermatitis producing Schistosome cercariae from Cultus Lake, British Columbia." *Canad. J. Res. (Zool.)*, **33,** 182–194. (W.L. 5898c)

FAIN, A., 1953.,—"Contribution à l'étude des formes larvaires des Trematodes au Congo Belge et spécialement de la larve de *Schistosoma mansoni*." *Mem. Inst. colon. belge Sci. nat.*, **22,** 1–312. (W.L. 13246b)

————, 1955,—"Sur une furcocercaire du groupe 'Ocellata' produisant expérimentalement la 'dermatite des nageurs' à Astrida (Runda-Urundi)." *Ann. Soc. belge Méd. trop.*, **35,** 701–707. (W.L. 926)

ILES, C., 1959.—"The larval Trematodes of certain freshwater molluscs. I. The Furcocercariae." *Parasitology*, **49,** 478–504. (W.L. 16035)

JOHNSTON, T. H. and CLELAND, E. R., 1937—"Larval trematodes from Australian terrestrial and fresh-water molluscs. Part II. *Cercaria* (*Furcocercaria*) *jaenschi* n.sp." *Trans. roy. Soc. S. Aust.*, **61,** 202, 206. (W.L. 21654)

———— and SIMPSON, E. R., 1939.—"Larval trematodes from Australian terrestrial and fresh-water molluscs. Part V." *Ibid.*, **63,** 63–68.

KOMIYA, Y. and ITO, J., 1952.—"The morphology of *Cercariae sturniae* Tanabe, 1948 (Cercaria of *Gigantobilharzia sturniae* (Tanabe, 1951)) a cause of cercaria dermatitis in Japan." *Jap. J. Med. Sci. Biol.*, **5,** 215–220.

LA VALETTE ST. GEORGE, A. J. H., 1855.—Symbolae ad trematodum evolutionis historiam. Berolini.

MATHESON, C., 1930.—"Notes on *Cercaria elvae* Miller as the probable cause of an outbreak of dermatitis at Cardiff." *Trans. R. Soc. trop. Med. Hyg.*, **25,** 421–424. (W.L. 21671)

MACFARLANE, W. V., 1944.—"Schistosome dermatitis in Southern lakes. An investigation of swimmer's itch." *N. Z. med. J.*, **43,** 136–140. (W.L. 15109)

———— and MACY, R. W., 1946.—"*Cercaria oregonensis* n.sp. a dermatitis-producing Schistosome cercaria from the Pacific North West." *J. Parasit.*, **32,** 281–285. (W.L. 11428)

MCLEOD, J. A., 1940.—"Studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba." *Canad. J. Res.*, (Sect. C.), **18,** 1–28. (W.L. 5898c)

MCMULLEN, D. B. and BEAVER, P. C. 1945.—"Studies on Schistosome dermatitis. IX. The life cycles of three dermatitis-producing Schistosomes from birds and a discussion of the sub-family Bilharziellinae (Trematoda : Schistosomatidae)." *Amer. J. Hyg.*, **42,** 128–154. (W.L. 600a)

MEYER, P. O. and DUBOIS, G. 1954.—"Dermatite humaine causée par des furcocercaires ocellées dans les bains publics de Zurich." *Bull. Soc. neuchâtel Sci. nat.*, **77,** 81–110. (W.L. 5299)

MILLER, Jr. H. M., 1923.—"Notes on some furcocercous larval trematodes". *J. Parasit.*, **11,** 35–46. (W.L. 11428)

————, 1926.—"Comparative studies on furcocercous cercariae III." *Illinois biol. Mongr.*, **10,** 1–112. (W.L. 9822)

————, 1927.—"Furcocercous larval trematodes from San Juan Island, Washington." *Parasitology*, **19,** 61–83. (W.L. 16035)

———— and NORTHUP, F. E., 1926.—"The seasonal infestation of *Nassa obsoleta* (Say) with larval trematodes." *Biol. Bull., Wood's Hole*, **50,** 490–508. (W.L. 2975)

NEUHAUS, W., 1952a.—"Biologie und Entwicklung von *Trichobilharzia szidati* n.sp. (Trematoda ; Schistosomatidae), einem Erreger von Dermatitis beim Menschen." *Z. Parasitenk*, **15,** 203–266. (W.L. 23536b)

————, 1952b.—"Der Einfluss des Zurischenwirtes auf die Gestalt der Cercariae von *Trichobilharzia szidati* Neuhaus, 1951 und ihre systematische Kenneichnung." *Zool. Anz.*, 148, 275–285. (W.L. 23833)

REES, G. F., 1932.—"An investigation into the occurrence, structure and life histories of the trematode parasites of four species of *Limnaea*." *Proc. zool. Soc. Lond.*, **1,** 1–32. (W.L. 16969b)

SEWELL, R. B. S., 1922.—"*Cercariae Indicae*." *Indian J. med. Res.*, **10,** suppl. pp. 1–370. (W.L. 9940)

SZIDAT, L., 1929.—"Zur Entwicklungsgeschichte der Bluttrematoden der Enten, *Bilharziella polonica* Kow. I. Morphologie und Biologie der Cercaria von *B. polonica* Kow." *Zbl. Bakt.*, **1,** III, 461–470. (W.L. 23684)

————, 1942.—"Was ist *C. ocellata* La Valette ? Morphologische und Entwicklungsgeschichtliche Untersuchungen uber der Erreger der europaischen Cercarien-Dermatitis des Menschen. 1." *Dtsch. tropen. med. Z.*, **46,** (19) 481–497. (W.L. 7327a)

————, 1942a.—"Idem II." *Ibid.*, **46** (20), 509–524.

————, and WIGAND, R. 1934.—Leitfaden der einheimischen Wurnkrankheiten des Menschen. *Verlage G. Thieme, Leipzig.*

TALBOT, S. N., 1936.—"Studies on Schistome dermatitis, II. Morphology and life history studies on three dermatitis-producing Schistosome cercariae, *C. elvae*, Miller, 1923, *C. stagnicolae* n.sp. and *C. physallae* n.sp." *Amer. J. Hyg.*, **23,** 372–384. (W.L. 600a)

TAYLOR, E. L. and BAYLIS, H. A., 1931.—"Observation and experiments on a dermatitis-producing cercaria and another cercaria from *Lymnaea stagnalis* in Great Britain." *Trans. R. Soc. trop. Med. Hyg.*, **24,** 219–244. (W.L. 21671)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 285-300.

The Behaviour and Development of *Brugia patei* (Buckley, Nelson and Heisch, 1958) in a Mosquito Host, *Mansonia uniformis* (Theobald)

By B. R. Laurence and F. R. N. Pester
London School of Hygiene and Tropical Medicine

The genus *Brugia* has been separated recently from *Wuchereria* by Buckley (1960) and, at present, contains three species, *B. malayi* (Brug, 1927), *B. pahangi* (Buckley & Edeson, 1956) and *B. patei* (Buckley, Nelson & Heisch, 1958). All three nematodes are found in both wild and domestic animals and all three have been found as parasites of the domestic cat. So far as is known, only *B. malayi* is normally a parasite of man, although an apparent infection of *B. pahangi* has been established in man experimentally (Edeson, Wilson, Wharton & Laing, 1960).

Cats infected with *B. patei* and *B. pahangi* have been maintained at the London School of Hygiene and Tropical Medicine for two and four years, respectively,* and the vector mosquito has been reared by the techniques already described by Laurence and Smith (1958). The infection of *B. patei* was first obtained from mosquitoes (*M. uniformis* and *M. africanus*) infected from cats in East Africa, brought to London, and then dissected for the infective stages. These were then inoculated by syringe into cats from England. The infections of *B. pahangi* were obtained by feeding laboratory reared mosquitoes on an infected cat brought to London from Malaya, and subsequent dissection for the infective stages and inoculation of these, by syringe, into fresh animals. The mosquito colonies originated from egg masses flown by air to London from Ceylon and Malaya. The behaviour of *B. patei* in the mosquito is described here, but some reference is made to infections with *B. pahangi* in the same mosquito for comparison.

Methods

The usual method used to infect mosquitoes was by laying an infected cat, anaesthetised with Nembutal, across the top of a 25 cm. cube cage containing hungry female *Mansonia*. To facilitate

*We are grateful to Professor J. J. C. Buckley, who first made these infections available to us for the experiments described in this paper. Mr. P. F. Blackburn has assisted us in maintaining the infections in the cats.

feeding by the mosquitoes, the fur was clipped from a small part of the side of the cat's body placed on the cage. This method of feeding is preferable to feeding the mosquitoes in small containers placed on the body of the infected animal, as there is no need to handle the gorged females in the cage after the feed. They then remain undisturbed in the cage until dissected. At all times the mosquitoes were kept in an insectary normally at 28°C. (26°–30°C.) and the humidity was maintained in the cages by covering part of the cage with damp lint. After feeding, the cage and lint were covered by polythene sheeting and this arrangement maintained the relative humidity in the cage above 70%.

Infected mosquitoes have been dissected in normal saline, after being stunned in a test tube, and the living larvae dissected out ; or the mosquitoes have been killed in warm 80% alcohol and stained in Mayer's acid haemalum for 3–6 days at 28°C., differentiated in tap water for 1–2 days and then dissected—a modification of Lebied's (1950) method for staining filarial larvae *in situ*, similar to that described by Nelson (1959).

Entry of the Microfilariae into the Mosquito

Mosquitoes were fed, at 10.30 a.m., on an anaesthetised cat which showed 100 or more microfilariae per 20–30 cu. mm. of peripheral blood three days before the experiment. The gorged mosquitoes were collected up in batches of 5 at intervals from 2 to 360 minutes after leaving the host. As the mosquitoes left the host over a period of about 10 minutes, allowance was made for this time interval when estimating the time after feeding. Each batch of 5 mosquitoes was killed in 80% alcohol, in tubes labelled with the time of collection after engorgement, and later stained with Mayer's acid haemalum. The head, thorax and abdomen were first separated, and then the blood mass in the stomach was removed by peeling away the rest of the abdominal tissue surrounding it. The blood mass, abdomen, thorax and head were then teased out separately in glycerin and examined for microfilariae, at magnifications of $\times 48$ and $\times 120$. The higher magnification was needed especially when searching tissue, such as the abdominal wall and fat body, for microfilariae.

The results of these dissections, expressed as percentages of the total microfilariae in the stomach (blood mass), abdomen and thorax at each time, are shown in Fig. 1. There is a steady penetration out from the stomach into the abdomen during the first 50 minutes following engorgement, and in this time approximately

80% of the microfilariae ingested escape from the blood in the stomach. Penetration of the stomach wall after this time then continues at a slower rate, but nearly all the microfilariae have escaped from the gut within 2 hours of engorgement. They appear in the abdomen outside the stomach within 2 minutes of the mosquito ceasing to feed and the number of microfilariae in the abdomen steadily increases during the first 50 minutes, the subsequent decline

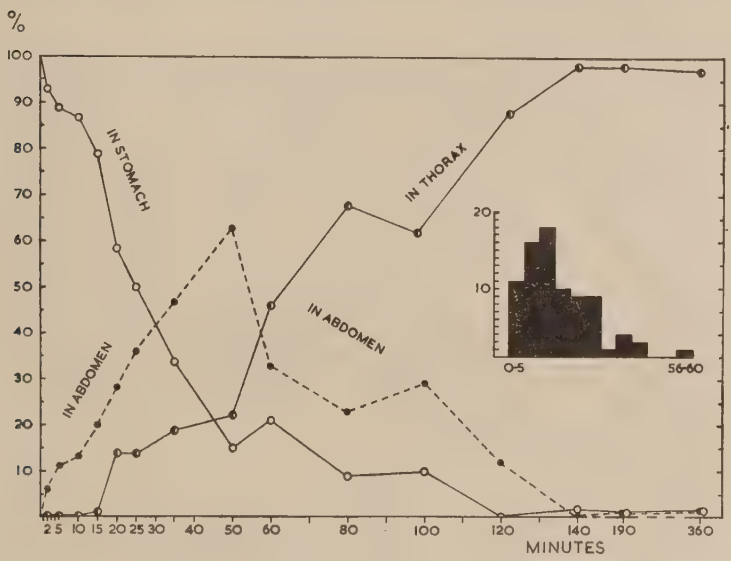


Fig. 1.—Percentages of the microfilariae of *B. patei* found in the stomach, abdomen and thorax of *M. uniformis* 2 to 360 minutes after a blood meal. The histogram shows the number of mosquitoes taking in 0–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35, 36–40, 41–45, 46–50, 51–55 and 56–60 microfilariae at this single feed.

in numbers in the abdomen after this time being due to the majority of them having then moved forwards to the thorax. This penetration into the thorax proceeds at a regular rate, about 15% of the microfilariae arriving in the thorax every 20 minutes, from 15 minutes after engorgement when the first microfilariae arrive, to 140 minutes when nearly all of them have settled in the thoracic indirect flight muscles.

Two results were at variance with this description and in Fig. 1 these results, at 30 and 40 minutes after engorgement, are pooled. Both these results were each weighted by 2 mosquitoes out of 5, each containing more than 25 microfilariae at an advanced stage of migration at 30 minutes and at a retarded stage of migration at 40 minutes. This gave percentage infections of the stomach, abdomen and thorax of 21%, 55% and 24% at 30 minutes, and 45%, 40% and 15% at 40 minutes.

There was no evidence that the microfilariae were more difficult to find in the mosquito at certain stages during this migration from the stomach to the thorax. The number ingested per mosquito at this single feed on one host varied from 1 to 58 (see Fig. 1), with an average intake of 16·5 per mosquito. When each batch of 5 mosquitoes was considered separately the average intake in each batch was from 9·6 to 24·0 microfilariae per mosquito and there was no obvious trend to indicate that they were more difficult to find when they were in, say, the thoracic muscles than when they were in the fat body or stomach. Neither was there any evidence of loss during the migration from stomach to thorax, other than 1 or 2 microfilariae remaining in the stomach, in the cercus or in the head. The mean number per mosquito in 5 mosquitoes killed at 2 minutes was 14·4 (range 6–20) compared with a mean of 12·4 (range 4–22) per mosquito at 6 hours. Wharton (1957a) also found no evidence of loss of microfilariae of *B. malayi* during the migration from stomach to thorax in *M. longipalpis*.

The Route of Migration from the Stomach to the Thorax

The dissections described above provided a method for determining the position of many of the microfilariae during their migration through the mosquito, and for tracing the route of the migration. Sheathed forms were found in the blood mass from the stomach from 2 to 100 minutes after engorgement. Only three sheathed microfilariae were found outside the stomach; one of these was in the fat body in the abdomen at 20 minutes after engorgement and the others were found at 60 minutes free in the abdomen, one completely and the other partially sheathed. Microfilariae were found associated with tissue from the stomach wall (Plate I, Fig. 1), or in stomach contents outside the peritrophic membrane, from 2 to 120 minutes after engorgement, which suggests that this is the period of penetration through the stomach wall (see also Fig. 1). A single microfilaria found embedded in the stomach wall 6 hours after engorgement may have died in this position.

Outside the stomach, many microfilariae have been recovered with part, or the whole, of the body penetrating cells of the fat body in the abdomen. The most usual place to find them in the abdomen was in, or near, fat cells lying close to the intersegmental muscles, or between these muscles and the body wall. They were also found alongside, or in, the heart in 5 mosquitoes and penetration of the heart may account for the rapid movement of some microfilariae forwards into the thorax.

Groups of 2 or more microfilariae, lying side by side or across one another in the tissues, were found in 14 of the mosquitoes dissected at a time when they were migrating through the abdomen. In 7 of these groups, 5–8 microfilariae were visible within the same microscopic field at a magnification of ×120, and these groups accounted for 52% of 84 microfilariae migrating in the abdomen of these mosquitoes. These larger aggregations were all amongst fat body cells lying near the body wall and the intersegmental muscles. This grouping during the migration through the abdominal tissues may be due to the restriction of these tissues within the abdomen by the distension of the stomach by the blood meal. After engorgement there is very little space in the abdomen between the wall of the stomach and the body wall. One consequence of this restriction of the tissues outside the stomach is that the microfilariae come together and may, in fact, be limited to only a few pathways in the body during the movement from the abdomen to the thorax. Also the narrow junction between thorax and abdomen restricts the movement of microfilariae within the body and may account for the close association of many of them later in the thoracic musculature. Two were found lying side by side across one row of oenocytes along the heart in the thorax. In the muscles, they were often found close together, sometimes lying side by side.

Microfilariae that have penetrated the indirect flight muscles have straightened between the muscle fibres, although the posterior end still shows some sinuosity (Iyengar, 1936). Migrating forms in the thorax show considerable sinuosity and these have been found amongst fat body cells, the heart and amongst the leg muscles near the side of the thorax, from 15 to 190 minutes after engorgement. Some microfilariae have been killed whilst apparently trying to penetrate between blocks of leg muscle, but those that have straightened have only been found between the fibres of the indirect flight muscles from 20 minutes after engorgement to 360 minutes, when all the forms in the thorax had settled in the muscle.

Some microfilariae may lose their way in the mosquito during this migration and enter the head, and one was found curled up in the eighth sternite at the tip of the abdomen 80 minutes after engorgement. Five mosquitoes, killed at 25, 60 and 140 minutes, contained microfilariae in the head. The early arrival of 2 specimens in the head of one mosquito within 25 minutes suggests that the route of migration here may have been within the heart and aorta.

Growth Within the Mosquito Host

Once the microfilariae of *B. patei* have penetrated into the indirect flight muscle and straightened between the muscle fibres, they shorten and thicken (Fig. 2a and Plate I, Fig. 2). A few active microfilariae of *B. patei* and *B. pahangi*, have been dissected out from the thorax of the mosquito 66 hours after the infective feed although, at this time, most of them have shortened and commenced to thicken. Some microfilariae do not throw off their sheaths completely before entering the thorax and may retain the sheath over the posterior part of the body. In these the anterior part, but not the posterior enclosed part, thickens, leaving the posterior end enclosed within the sheath with the appearance of an appendix to the worm. This retention of the sheath and subsequent malformation during growth has also been figured in *B. malayi* by Feng (1936). When flight muscle containing the thickened, but not elongated forms, (the "sausage stage") is stained in Mayer's haemalum these larvae are found to lie inside well defined cavities in the flight muscle, which have the appearance of being digested out of the muscle surrounding them (Plate I, Fig. 2). These cavities are smooth in outline around the worm's body except at the posterior end where the tail, which can be moved by the larvae, apparently triturates the muscle at the posterior end (Plate I, Fig. 2d). These cavities are not found around the microfilariae in the muscle, fixed and stained in the same way. Lebied (1960) has illustrated similar cavities in the muscles of *Simulium* surrounding the "sausage stage" larvae of *Onchocerca*.

Eventually the worms begin to grow in length, usually on the third or fourth day after the day of infection (Fig. 2b) and elongate rapidly along the axis of the muscle fibres in which they lie. When microfilariae have settled in the muscle close together, the developing larvae may elongate along the same muscle line but the worms double back from one another, although the cavities in which they lie may then become confluent. Although there may be differences

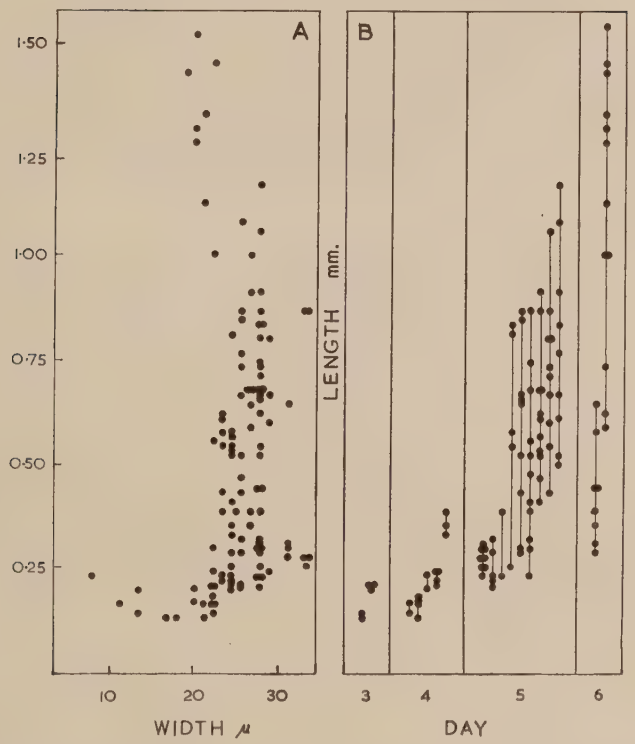


Fig. 2a.—Growth of *B. patei* in *M. uniformis*, showing shortening and thickening, followed by elongation.

Fig. 2b.—Growth of *B. patei* in *M. uniformis*, showing variation in growth of larvae between individual mosquitoes on the third, fourth, fifth and sixth days after infection. Each line represents one mosquito and connects measurements of the lengths of larvae found in these mosquitoes.

in the stage of growth reached between individual larvae in the same mosquito, especially during the stage of rapid elongation, generally all the larvae in one mosquito tend to grow together. However, between individual mosquitoes there are often considerable difference in the stage of growth attained, (Fig. 2b), although the mosquitoes are fed at the same time and the microfilariae arrive in the thorax within 2 hours of the blood meal (Fig. 1).

Structural changes during growth are generally similar to those described for *B. malayi* by Feng (1936). Worms moulting into the second stage have been found on the fourth day after the infective feed, and second stage and third stage larvae have been found together on the sixth day after infection. In one experiment 44% of the 550 worms recovered from 14 mosquitoes had reached the infective stage on the eighth day after infection, whereas one and two days later 84–85% of the 670 worms recovered from 20 mosquitoes were infective.

This development of *B. patei*, under the same conditions, is more rapid than that of *B. pahangi* in which species the larvae are not infective until at least the ninth day after the infective feed and where 81–83% of the larvae were found to be infective on the tenth and eleventh days after infection. The rate of development of *B. malayi* in *Mansonia* is similar to that of *B. pahangi* and Wharton (1957b) found 65% of the larvae mature by the eleventh day.

Although the rate of development may vary, most of the microfilariae ingested appear to be capable of development to the infective stage. Mosquitoes infected with *B. patei* were killed at intervals during the development to the infective stage. The number of larvae per mosquito varied from 19–26, 8–23, 8–58 and 26–45 on the third, fourth, fifth and sixth days, respectively, after infection. With *B. pahangi*, the mean number of larvae recorded per infected mosquito between the third and sixth days after feeding was 13·8 (range 1–30) compared with 12·1 per mosquito (range 1–60) recorded on days seven to eleven. Wharton (1957a) also found no evidence of loss of worms during the development of *B. malayi* in *M. longipalpis* although, in all three species of *Brugia*, some microfilariae are unable to rid themselves of the microfilarial sheath and then fail to develop normally. In *M. uniformis* these individuals are rare.

The Infective Stage

Larvae in the infective stage are more active than the earlier stages and migrate out from the thorax into the rest of the body. There is a marked concentration of the infective larvae of both *B. patei* and *B. pahangi* in the head and proboscis of the mosquito ; approximately three times the number are found here compared with the number found in the abdomen (Fig. 3). Out of 1,857 infective stage larvae of *B. patei* recovered from *M. uniformis*, 53% had migrated into the head and proboscis and 19% into the abdomen.

A similar distribution of the infective stages was found in *B. pahangi*, where 65% of 1,339 infective larvae were recovered from the head and proboscis, compared with 12% in the abdomen. Up to 44 infective larvae of *B. patei* have been recovered from the head and proboscis of the mosquito and, when large numbers are present in the head, the proboscis and neck are markedly distended with the closely packed larvae.

The results from different feeds of *M. uniformis* on animals infected with *B. patei* and *B. pahangi* are shown graphically in Fig. 3, where the numbers in the head and abdomen are compared with similar observations on the distribution of the infective larvae of *W. bancrofti* in the mosquitoes *Culex* (Hu & Chang, 1933 ; Hu & Yen, 1933 ; Giglioli, 1949 ; Jordan, 1959 ; Omori, 1958 ; Pratt & Newton, 1946), *Aedes* (Hicks, 1932) and *Anopheles* (Giglioli, 1949) ; *A. gambiae* (Pester, unpublished) at temperatures above 16°C. Although this difference in the distribution of the infective stages of *Brugia* and *Wuchereria* may be due to differences in the mosquito host, or even laboratory technique, this concentration of the infective larvae of *Brugia* in the head and proboscis of the mosquito may well prove to have some epidemiological significance.

Certainly, females of *Mansonia* can carry a heavy load of the infective stages of *Brugia*. When batches of mosquitoes were fed on the same cat infected with *B. patei*, between December 1958 and June 1959, the average infection over this period was 22 infective larvae per mosquito of 85 mosquitoes that survived to the infective stage. In these mosquitoes 52% contained less than 20 infective larvae, yet the remaining 48% survived equally well with 20 or more per mosquito. In this series the maximum number recovered from one mosquito was 69. Wharton (1957b) has published similar figures of high densities of infective larvae of *B. malayi* in *M. longipalpis* infected in the laboratory in Malaya.

Discussion

Wharton (1957b) has already shown that *M. longipalpis* is a remarkably efficient vector of *B. malayi* in that the mean number of microfilariae ingested was about five times the apparent number present per cu. mm. in the peripheral blood of the vertebrate host, although the blood meal taken was less than 5 cu. mm. The evidence presented here for *B. patei* shows that most, about 98%, of the microfilariae ingested by *Mansonia* successfully migrate into the

thoracic muscles of the invertebrate host and that in *B. patei*, and also in *B. pahangi*, 80% or more (mean 85·4%, range 68–96%) of the worms develop to the infective stage in the mosquito. There is little evidence of any considerable loss in number during the development within the mosquito host. Wharton (1957a) has given rather similar figures for semi-periodic *B. malayi* in *M. longipalpis*.

Wharton (1957b), however, recorded that when large numbers of larvae of *B. malayi* are present in *M. longipalpis*, e.g. more than a mean number of 80 per mosquito, the development of most of them is arrested and only about 10–12% mature in the normal time. The present observations were on mosquitoes infected with mean numbers per batch of mosquitoes of 16–40 per mosquito. With infections of this order, 100% of the mosquitoes fed on cats infected with *B. patei* were found to be infected, and 90–100% of those fed on cats infected with *B. pahangi*. Wharton (1957b) found that 100% of the mosquitoes became infected when the mean number of larvae of *B. malayi* per mosquito was 8·3 or more, but that mosquitoes fed on carriers with 10·7 or more microfilariae per cu. mm. died more rapidly than those fed on hosts with a smaller microfilaraemia. *Mansonia* therefore appears to be most receptive to the three species of *Brugia* at densities of from 10 to 50 larvae per mosquito. At these densities, in the laboratory, the majority of the ingested microfilariae develop to the infective stage.

Of the worms that develop to the infective stage 50–65% become concentrated in the head and proboscis of the mosquito. This concentration takes place without any stimulus from the mosquito probing or feeding on blood. There is an apparent difference here in the behaviour of *Brugia* within the mosquito host compared with that of *Wuchereria bancrofti*, in which species the infective larvae are more equally distributed in the body. Pratt & Newton (1946) suggested that the infective larvae of *W. bancrofti* first migrate to the abdomen before entering the head although in both *Wuchereria* and *Brugia* they are capable of rapid movement through the body of the mosquito (see review by Lavoipierre, 1958). Despite the greater concentration of the infective larvae of *Brugia* in the head and proboscis of the mosquito, and the associated increased certainty of infection from *Mansonia* infected with this genus, all the infective larvae within the mosquito may be capable of migrating to the head and proboscis at the time of the feed (see Jordan, 1959).

There is a conflict of opinion as to the route of migration of the microfilariae of *Brugia* in *Mansonia* and, also, the rapidity with which they migrate from the blood meal into the thorax of the mosquito. Iyengar (1936) found the microfilariae of *B. malayi* in the cardiac (thoracic) part of the stomach of *M. annulifera* and concluded from the rapidity with which the microfilariae penetrated

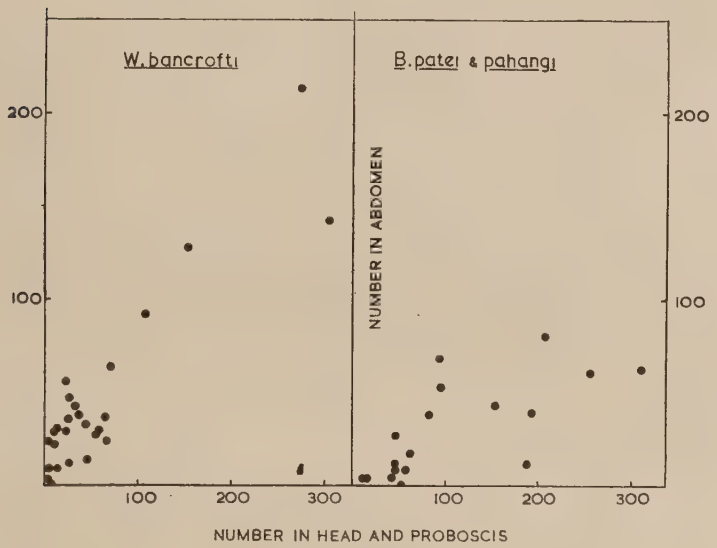


Fig. 3.—Comparison of the number of infective larvae of *Wuchereria* and *Brugia* recovered from the head and abdomen of mosquito hosts. Each point represents the total infection from one infective feed of several mosquitoes.

into the thoracic flight muscles, within two hours, that migration from the gut into the flight muscles took place through the wall of the cardiac stomach directly into the thorax. Wharton (1957a) found no microfilariae in the cardiac portion of the stomach of *M. longipalpis*, but found numbers in the stomach and, after 10 minutes, in the abdomen and in the thorax. The present observations show that the microfilariae of *B. patei* in *M. uniformis* migrate from the blood meal in the stomach into the abdomen, and then migrate

through the fat body and haemocoel forwards into the thorax (Fig. 1). This is accomplished, as Iyengar (1936) observed, within 2 hours of the blood meal. Lavoipierre (1958b) has reviewed the literature on the penetration of microfilariae from the gut of the arthropod host and concludes that penetration of the gut wall is usually prolonged over many hours. This has not been observed in *B. patei* in the mosquito host. In *M. uniformis*, and also in *Aedes togoi* Theobald, the microfilariae of *B. patei* penetrate out from the gut rapidly after ingestion. Microfilariae have not been found in numbers in the stomach of *M. uniformis* after the first 2 hours following the blood meal, although Iyengar (1936) and Wharton (1957a) record microfilariae of *B. malayi* in the stomach of *Mansonia* after 2 hours, in the latter case after feeding on a heavily infected host. Iyengar (1936) found that, although microfilariae were present in the stomach after 2 hours, there was then no increase in the number present in the haemocoel and thoracic muscles after this time. The rapid migration out from the stomach in *Mansonia* of the microfilariae of *Brugia* may be to avoid coagulation and digestion of the blood meal (Kartman, 1953), but does not appear to be due to limitations of food reserves in the microfilariae which might soon be expended and not replaced (Taylor, 1960). There is no thick peritrophic membrane in *Mansonia* comparable to that found in *Simulium* after a blood meal which, as Lewis (1953) has shown, may imprison the microfilariae of *Onchocerca* and prevent migration from the stomach.

As there is little delay in the migration of most of the microfilariae from the blood meal to the thoracic flight muscles, this cannot explain the variation found later in their development in the thoracic musculature (Fig. 2). The route along which they travel between the stomach and thorax, through the fat body, heart and haemocoel, is greatly restricted by the distension of the stomach by the blood meal and by the tracheal system (Lavoipierre, 1958) and this may account for the microfilariae being found close together during this migration and, also, later when once they have penetrated within the muscle bundles of the indirect flight muscles. They appear to penetrate and develop only in this fibrillar type of muscle, which is interesting as fibrillar muscle has unusual physiological properties, becoming deactivated by release and contracting rapidly following stretching. The underlying mechanisms of this, unfortunately, are not understood (Pringle, 1957). The microfilariae obviously digest away the muscle after they have shortened and thickened to the "sausage stage" and also presumably obtain nutriment from it during elongation to the infective stage.

A few microfilariae do not settle down to shorten and thicken in the muscle immediately but remain active and undeveloped up to three days after the infective feed. This may account for the variation in the stage of growth attained in the first three days after the blood meal ; but generally there is a much greater variation in the stage of growth reached by larvae in different mosquitoes than between those in the same mosquito, and this is independent of the number of larvae present. This suggests that the metabolism of the mosquito host may determine the rate of growth within the fibrillar flight muscles. Certainly the rate of digestion of the blood meal varies from one individual mosquito to another and, once the eggs are mature, so also does the amount of fat reserves left over in the fat body. At present the fate of the blood meal taken in by mosquitoes and other blood-sucking arthropods, is not properly understood, although it is well-known that fats and proteins, presumably from those meals, are concentrated into the oocytes and that this is under the control of an ovarian development hormone initiated from the head (Gillett, 1956). It is perhaps not completely coincidental that this developmental period of the eggs in the vector coincides with the time that the filarial larvae remain relatively poorly differentiated within the host. But the development of the filarial larvae is not dependent upon the metabolism normally associated with the development of the ovaries of the host. In four experiments 13 female *Mansonia* out of 34 failed to develop eggs, yet the development of *B. patei* to the infective stage was the same in all of these females. One female with undeveloped ovaries still retained the blood meal in the stomach 11 days after infection but contained completely developed infective larvae. Possibly the high concentration of free amino acids in the haemolymph of insects (Florkin, 1949) enables these arthropods, which feed in any case on a protein rich diet, to tolerate the high density of developing filarial larvae found in them but, at present, this relationship between the metabolism of the mosquito host and the parasite is not understood. Yet this might explain the tolerance of some species, or strains, of mosquito to infection and the intolerance of others. As Lebied (1960) has recently suggested, advances in knowledge in this direction require the development of new techniques.

Summary

1. The behaviour and development of *Brugia patei* has been followed in the mosquito host, *Mansonia uniformis*, from ingestion of the microfilariae to the development of the infective stages.

2. The microfilariae penetrate very rapidly out of the stomach into the abdomen of the mosquito; 80% of them escape from the stomach during the first 50 minutes after a blood meal.

3. The microfilariae migrate from the abdomen to the thorax through fat body cells and the heart during the first 140 minutes followed engorgement and begin to penetrate the indirect flight muscles within 15 minutes from engorgement. During this migration microfilariae were found in close association, suggesting that they follow similar pathways of migration through the body of the mosquito.

4. Most microfilariae (98%) have settled in the indirect flight muscles within 2 hours of engorgement. In these muscles the larvae digest away the muscle surrounding them but do not begin to elongate until the third or fourth day after the blood meal. The rate of development varies from one mosquito host to another, but about 80% of the larvae have developed to the infective stage by the ninth to tenth days after the infective feed. The infective stages concentrate in the head and proboscis of the mosquito vector. The development of *B. patei* is faster than that of *B. pahangi* in mosquitoes from the same colony.

5. The efficiency of *M. uniformis* as a vector of *B. patei* is compared with the similar efficiency of *M. longipalpis* as a vector of *B. malayi*. In both mosquitoes the majority of the microfilariae ingested develop to the infective stage. *M. uniformis* is also an efficient vector of *B. pahangi*.

6. The development of the parasite is discussed briefly with reference to knowledge of the physiological events within the mosquito host subsequent to the blood meal.

References

Buckley, J. J. C., 1960.—"On *Brugia* gen. nov. for *Wuchereria* spp. of the '*malayi*' group, i.e., *W. malayi* (Brug, 1927), *W. pahangi* Buckley and Edeson, 1956, and *W. patei* Buckley, Nelson and Heisch, 1958." *Ann. trop. Med. Parasit.*, **54**, 75–77. (W.L. 1063)

Edeson, J. F. B., Wilson, T., Wharton, R. H. and Laing, A. B. G., 1960—"Experimental transmission of *Brugia malayi* and *B. pahangi* to man." *Trans. R. Soc. trop. Med. Hyg.*, **54**, 229–234. (W.L. 21671)

Feng, L. C., 1936.—"The development of *Microfilaria malayi* in *A. hyrcanus* var. *sinensis* Wied." *Chin. med. J.*, Supp. 1, 345–367. (W.L. 617788)

Florkin, L. C., 1949.—Biochemical Evolution. Translated by S. Morgulis. Academic Press, New York.

Giglioli, G., 1948.—"The transmission of *Wuchereria bancrofti* by *Anopheles darlingi* in the American tropics." *Amer. J. trop. Med.*, **28**, 71–85. (W.L. 626)

Gillett, J. D., 1956.—"Initiation and promotion of ovarian development in the mosquito *Aëdes* (*Stegomyia*) *aegypti* (Linnaeus)." *Ann. trop. Med. Parasit.*, **50**, 375–380. (W.L. 1063)

Hicks, E. P., 1932.—"The transmission of *Wuchereria bancrofti* in Sierra Leone." *Ibid.*, **26**, 407–422.

Hu, S. M. K. and Chang, T. L., 1933.—"Observations on natural infection of *Culex pipiens* Linnaeus var. *pallens* Coquillett with *Wuchereria bancrofti* Cobbold in Woosung District, Shanghai, China." *Chin. med. J.*, **47**, 1367–1372. (W.L. 617788)

———, and Yen, C. H., 1933.—"Studies on the susceptibility of *Culex pipiens* Linnaeus var. *pallens* Coquillett to experimental infection with *Wuchereria bancrofti* Cobold in Shanghai area." *Ibid*, **47**, 1359–1366.

Iyengar, M. O. T., 1936.—"Entry of *Filaria* larvae into the body cavity of the mosquito." *Parasitology*, **28**, 190–194. (W.L. 16035)

Jordan, P., 1959.—"A note on the effect of a blood meal on infective larvae of *Wuchereria bancrofti* in *Culex fatigans*." *Trans. R. Soc. trop. Med. Hyg.*, **53**, 148–150. (W.L. 21671)

Kartman, L., 1953.—"Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856)." *Exp. Parasit.*, **2**, 27–78.

Laurence, B. R. and Smith, S. A., 1958.—"The breeding of *Taeniorhynchus* (subgenus *Mansonioides*) mosquitoes in the laboratory." *Trans. R. Soc. trop. Med. Hyg.*, **52**, 518–526. (W.L. 21671).

Lavoipierre, M. M. J., 1958.—"Studies on the host-parasite relationships of filarial nematodes and their Arthropod hosts. II. The Arthropod as a host to the Nematode : A brief appraisal of our present knowledge, based on a study of the more important literature from 1878 to 1957." *Ann. trop. Med. Parasit.*, **52**, 326–345. (W.L. 1063)

Lebied, B., 1950.—Une nouvelle théorie epidémiologique. Dijon : 54 pp.

———, 1960.—"Mise au point à propos d'une nouvelle méthode de dissection des vecteurs des filarioses. Le but de cette méthode." *Riv. Parassit.*, **21**, 71–79. (W.L. 19442a)

Lewis, D. J., 1953.—"*Simulium damnosum* and its relation to onchocerciasis in the Anglo-Egyptian Sudan." *Bull. ent. Res.*, **43**, 597–644. (W.L. 4184)

Nelson, G. S., 1959.—"The identification of infective filarial larvae in mosquitoes: with a note on the species found in 'wild' mosquitoes on the Kenya coast." *J. Helminth.*, **33**, 233–256. (W.L. 11224a)

Omori, N., 1958.—"Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of Bancroftian filariasis. 5. On the distribution of infective larvae in mosquito and the effect of parasitism of filariae upon the host insect." *Nagasaki med. J.*, **33,** 143–155.

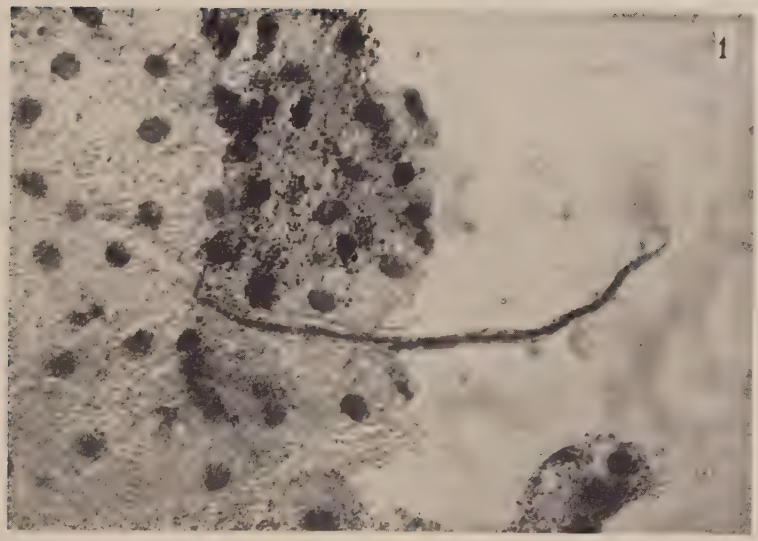
Pratt, I. and Newton, W. L., 1946.—"The migration of infective larvae of *Wuchereria bancrofti* within the mosquito host and their rate of escape under laboratory conditions." *J. Parasit.*, **32,** 272–280. (W.L. 11428)

Pringle, J. W. S., 1957.—Insect Flight. University Press, Cambridge.

Taylor, A. E. R., 1960.—"Maintenance of filarial larvae *in vitro*." *Exp. Parasit.*, **9,** 113–120.

Wharton, R. H., 1957a.—"Studies on filariasis in Malaya : Observations on the development of *Wuchereria malayi* in *Mansonia* (*Mansonioides*) *longipalpis*." *Ann. trop. Med. Parasit.*, **51,** 278–296. (W.L. 1063)

————, 1957b.—"Studies on filariasis in Malaya : The efficiency of *Mansonia longipalpis* as an experimental vector of *Wuchereria malayi*." *Ibid.*, **51,** 422–439.



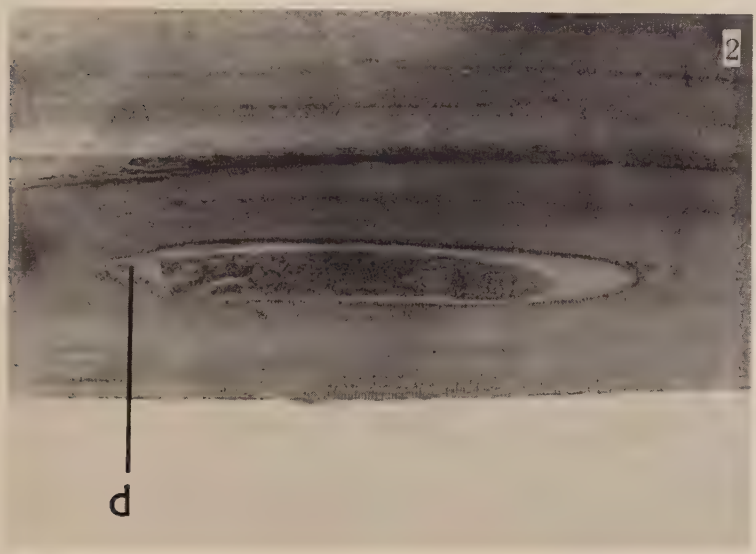


Fig. 1.—Photograph of microfilaria penetrating stomach wall 2 minutes after the engorgement of the mosquito. Fat cells, on the outside of the stomach, are heavily pigmented. (Stain : Mayer's acid haemalum. Photograph : S. A. Smith.)

Fig. 2.—Photograph of "sausage stage" larva of *B. patei* to show damage to the mosquito flight muscle. Damaged muscle from movements of the tail is labelled **d.** (Stain : Mayer's acid haemalum. Photograph : S. A. Smith.)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 301–304.

Elongoparorchis pneumatis gen. et sp. nov. (Trematoda) from the Air-bladder of the Marine Catfish, *Arius jella* (Val.)

By K. Hanumantha Rao, D.Sc.*

From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

In the course of investigations on the helminth parasites of marine fishes of the coastal waters at Waltair (India) it was observed that an apparently hitherto undescribed trematode occurred in the air-bladder of the catfish, *Arius jella*. Previous records of air-bladder trematodes are those of Southwell (1913) who reported the genus *Isoparorchis* from several freshwater fishes and *Cylindrorchis tenuicutis* from the marine fish, *Tetrodon stellatus*, at Ceylon. The present fluke whilst bearing a very superficial resemblance to *Cylindrorchis* is closely related to *Isoparorchis* but it differs from the latter in some important respects which appear to have generic value. For this reason a new genus and species *Elongoparorchis pneumatis* is proposed for the present form from *Arius jella*.

Material and Methods

The specimens on removal from the host were relaxed in tap water and fixed in 5% formalin, 70% alcohol or Bouin's fluid. Whole mounts were stained with alum carmine. Alcohol fixed specimens were treated with Fast Red Salt B or Catechol before staining with alum carmine. Sections 8–12μ thick were stained with Ehrlich's haematoxylin and eosin or PAS-light green methods.

Observations

In life the translucent worms appear light brown in colour showing slight movements on the silvery white wall of the air-bladder. Brown pigment trails presumably of haematin are often left on the wall,

* Permanent address : Department of Zoology, Andhra University, Waltair, S. India.

hence the presence or absence of the parasites could immediately be ascertained on opening the bladder. A full grown specimen (Fig. 1) has the following measurements. Length is 9·2 mm. with greatest breadth of 3·5 mm. The subterminal oral sucker (OS) is 0·6 mm. in diameter ; the acetabulum (AC) situated 2 mm. from the anterior end, is 0·77 mm. across. The genital pore is situated immediately behind the oral sucker. The pharynx (PH) measuring $0{\cdot}2 \times 0{\cdot}17$ mm. opens into a thick walled glandular stomach (GS) which opens into the intestinal caeca. The caeca run in a sinuous course and reach the posterior end. The testes which lie dorsal to the intestinal caeca are symmetrical and situated postero-lateral to the acetabulum. Each testis (T) is elongated, arcuate with dilatations or may be sinuous and cylindrical. The vasa deferentia meet in a vesicula seminalis (VS) followed by a pars prostatica (PP) and the ejaculatory duct which finally opens into a hermaphroditic sac. The female reproductive apparatus is situated in the posterior region of the body. The ovary (OV) lies on the left side and consists of four club-shaped lobulated arms. The short oviduct is united by a duct from the conspicuous receptaculum seminis (RS). The vitellaria (VT) are arranged in two groups of three lobulated radiating branches on the right and four on the left, situated behind the ovary and the Mehlis's gland complex. The vitelline ducts from the two groups unite and the median duct opens into the ootype near the junction of the oviduct and the duct from the receptaculum seminis. The thick walled ootype is surrounded by prominent Mehlis's gland cells (MG). The ootype continues into the uterus which ascends up following a winding course occupying the preovarian region and finally opening into the hermaphroditic sac. Eggs are small, measuring $13{\cdot}2\mu \times 6{\cdot}6\mu$. Excretory stem is sigmoid with a posterior vesicle.

Discussion

In the general morphology and anatomy, especially the presence of a glandular stomach, long serpentine caeca, postero-lateral position of the symmetrically arranged testes and the position and arrangement of the female reproductive apparatus, the present trematode resembles the genus *Isoparorchis* Southwell, 1913, and the characters suggest that it should belong to the family Isoparorchiidae Poche, 1926, (see Yamaguti, 1958). But as it becomes obvious from the table of characters given below the present trematode to which the new generic name *Elongoparorchis* is given, appreciably differs from the genus *Isoparorchis*.

Isoparorchis Southwell, 1913	*Elongoparorchis* n.g.
1. Testes round.	1. Testes elongated sinuous and cylindrical or arcuate with dilatations.
2. Ovary band shaped.	2. Ovary branched with club shaped lobulated arms.
3. Each group of vitellaria dendritic.	3. Each group of vitellaria with radiating lobulated arms.

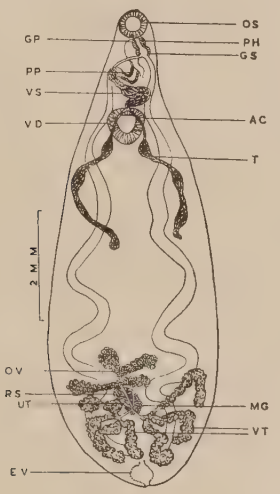


Fig. 1.—*Elongoparorchis pneumatis* gen. et sp. nov., dorsal view.

AC=Acetabulum ; **EV**=Excretory vesicle ; **GP**=Genital pore ; **GS**=Glandular stomach ; **MG**=Mehlis's gland ; **OS**=Oral sucker ; **OV**=Ovary ; **PP**=Pars prostatica ; **RS**=Receptaculum seminis ; **T**=Testis ; **UT**=Uterus ; **VD**=Vas deferens ; **VS**=Vesicula seminalis ; **VT**=Vitellarium.

Hence it would appear that the present trematode requires to be separated from *Isoparorchis* and described as constituting a new genus and species.

Diagnosis of *Elongoparorchis pneumatis* gen. et sp. nov.

Isoparorchiidae with foliate translucent light brown body. The subterminal oral sucker slightly smaller than the acetabulum. Genital pore median close behind the oral sucker. Muscular pharynx followed by a glandular stomach leading into sinuous caeca reaching the posterior end. Testes postero-lateral to acetabulum, elongated, sinuous, cylindrical or arcuate with dilatations. Ovary branched with lobulated club shaped arms situated on the left side. Vitellaria in two groups, each group with three or four radiating lobulated arms situated posterior to the ovary and the Mehlis's gland complex in the posterior region of the body. Receptaculum seminis large, on left side. Uterus extensively coiled occupying the pre-ovarian region. Hermaphroditic sac conspicuous. Eggs small. Excretory stem sigmoid with a posterior vesicle. Parasitic in the air-bladder of marine fishes.

Elongoparorchis pneumatis gen. et sp. nov.
Host : *Arius jella* (Val.)
Habitat : Air-bladder.
Locality : Waltair, India.

Summary

1. A new genus and species of Isoparorchiidae Poche, 1926, is described from the air-bladder of the marine catfish, *Arius jella* (Val.), caught off the coast of Waltair, India. The new fluke is named *Elongoparorchis pneumatis*.

2. The new genus *Elongoparorchis* bears a superficial resemblance to the genus *Isoparorchis* Southwell, 1913, but differs appreciably in the structure of the testes, the ovary and the vitellaria to warrant the separation.

Acknowledgments

Thanks are expressed to the Government of United Kingdom for a Scholarship under the Colombo Plan. Grateful thanks are due to Professor J. J. C. Buckley for facilities, interest and encouragement. My thanks are also due to Mr. S. Prudhoe (British Museum) for helpful suggestions.

References

Southwell, T., 1913.—"Notes from the Bengal Fisheries Laboratory, Indian Museum. No. 1." *Rec. Ind. Mus.*, **9**, 79–103. (W.L. 17746)
Yamaguti, S., 1958.—Systema Helminthum. Vol. 1. The Digenetic trematodes of vertebrates. Interscience Pub. Inc., New York.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 305–308.

Pulmoverminae n.subfam. (Trematoda: Digenea) for the Genera *Pulmovermis* and *Hydrophitrema*

By Dorothea F. Sandars*
Queensland Institute of Medical Research

On 18th August 1960, a description of a digenetic trematode, *Pulmovermis cyanovitellosus* Coil and Kuntz, from the lungs of a sea-snake, *Laticauda semifasciata* (Reinwardt, 1837), from Yeh Yu, was published. On 20th August 1960, a description of a similar worm, *Hydrophitrema gigantica* Sandars, from a sea-snake, *Hydrophis elegans* (Gray, 1842), from Moreton Bay, South Queensland, appeared. It seemed that these two genera might by synonymous, but a comparison of the type specimens showed that they may be accepted as two distinct genera, closely related, and neither fitting into any of the present subfamilies of the Hemiuridae Lühe, 1901. A new subfamily, Pulmoverminae, is therefore proposed to receive them.

Pulmoverminae n.subfam.

Subfamily diagnosis : Hemiuridae : Body elongate with short ecsoma (tail). Cuticle with or without spines. Intestinal caeca extend almost to posterior extremity of body. Acetabulum near anterior extremity. Testes post-acetabular. Seminal vesicle tubular with heavy muscular walls, may extend from hindbody into forebody. Ductus hermaphroditicus enclosed in a pouch. Cirrus pouch absent. Ovary post-acetabular. Vitellaria in two compact, lobed masses, immediately post-ovarian. Uterus extending almost to posterior extremity of body. Excretory arms united anteriorly. Parasites in lungs of sea-snakes.

The Pulmoverminae may be distinguished from the Sterrhurinae, the Dinurinae, the Hemiurinae and the Dissosaccinae by the position of the long seminal vesicle which may extend from the hindbody into the forebody. It is also different from the Sterrhurinae and the Dinurinae in having compact vitellaria, and from the Hemiurinae because of the presence of an hermaphroditic pouch which is not "strongly muscular" as it is in the Dissosaccinae.

* Also of the Department of Medicine, University of Queensland.

Genera : **Pulmovermis** Coil and Kuntz, 1960

Hydrophitrema Sandars, 1960

The generic diagnosis of *Hydrophitrema* is revised so as to delete "without tail". In the specimens examined it is difficult to determine with certainty whether an ecsoma is present. In the original description it was stated to be absent, although it was realized that possibly the distinct, but small, posterior tapering of the body could be an everted ecsoma. In view of the fact that *Pulmovermis* and *Hydrophitrema* are closely related genera having a number of features in common, and as there is a definite and relatively small ecsoma present in *Pulmovermis*, it is now considered that the distinct tapering of the posterior body represents a small ecsoma in *Hydrophitrema*. An examination of further specimens will be necessary to decide this.

Comparison

Pulmovermis and *Hydrophitrema* are both elongate worms in which the acetabulum is close to the oral sucker, there is no oesophagus, and the vesicula seminalis is large with heavily muscular walls ; the rest of the reproductive organs lie in the same relative positions in both.

Pulmovermis and *Hydrophitrema* are distinguished by the following features :—

(a) The body shapes are different, *Pulmovermis* being much smaller and more slender than *Hydrophitrema* ; the body length of *Hydrophitrema* (170–260 mm.) is more than ten times that of *Pulmovermis* (up to 17 mm.).

(b) The acetabulum in *Pulmovermis* is approximately one-ninth of the total body length from the anterior end, whereas in *Hydrophitrema* it is approximately one quarter ; in *Pulmovermis* it appears to be pedunculate, and the sides of the anterior part of the body curve ventrally, which, together with a distinctly ventrally directed oral sucker, gives a very characteristic appearance ; there is no evidence of any acetabular stalk in *Hydrophitrema*, and no ventral flexing of the anterior end of the body.

(c) The testes in *Pulmovermis* lie in tandem within the posterior half of the body and are well removed from the acetabulum ; in

Hydrophitrema the testes are obliquely tandem in the anterior half of the body and only a short distance behind the acetabulum.

(d) The ovary in *Pulmovermis* lies near the mid-line in the posterior quarter of the body, whereas in *Hydrophitrema* it is laterally placed and in the anterior half of the body.

(e) The vesicula seminalis of *Hydrophitrema* has a very much heavier muscular wall than in *Pulmovermis*, its length relative to the body length is much shorter (in *Hydrophitrema* 1 : 47 ; in *Pulmovermis* 1 : 2), and it extends anteriorly to the acetabulum ; in *Pulmovermis* the vesicula seminalis does not extend further forward than the level of the acetabulum.

The major features on which generic differentiation is based are : the relative positions of the acetabulum, testes and ovary ; the comparative lengths of the vesiculae seminales relative to the body lengths, and position of their anterior ends relative to the acetabulum.

Discussion

The erection of a new subfamily to receive *Pulmovermis* and *Hydrophitrema* seems necessary, as it is impossible to place either in any existing subfamily of the Hemiuridae.

Coil and Kuntz (1960) imply that they place *Pulmovermis* in the Hysterolecithinae, and state that it is "most similar to *Hysterolecitha* Linton, 1910". Although listing a number of differences from other genera in the Hysterolecithinae, they have apparently overlooked that one of the features of the Hysterolecithinae is the absence of a tail. *Pulmovermis* has a small, but quite definite, tail.

Sandars placed *Hydrophitrema* in the Derogenetinae, considering no tail to be present. If the decision that it has a small tail is correct, then it is not a member of the Derogenetinae.

The key by Yamaguti (1958) to the subfamilies of the Hemiuridae Lühe, 1901, is inadequate, as there is no provision for genera with a seminal vesicle which has a heavy muscular wall and may extend from the hindbody into the forebody. It can be modified by an additional alternative in couplet 6 (p. 261) to read "Seminal vesicle with heavy muscular wall, may extend from hindbody to forebody ; vitellaria compact . . . . . Pulmoverminae".

Summary

Pulmoverminae is proposed as a new subfamily within the Hemiuridae Lühe, 1901, to receive the genera *Pulmovermis* Coil and Kuntz, 1960 and *Hydrophitrema* Sandars, 1960. The genera are compared, and considered to be distinct.

Acknowledgments

I wish to thank Dr. A. C. Smith, Director of the Smithsonian Institution, U.S. National Museum, Washington, and Mr. G. Mack, Director of the Queensland Museum, Brisbane, whose kind co-operation made it possible to examine both type specimens. I am also grateful to Dr. E. C. y Caballero of Mexico for his helpful comments.

References

Coil, W. H. and Kuntz, R. E., 1960.—"Three New Genera of Trematodes from Pacific Sea Serpents, *Laticauda colubrina* and *L. semifasciata*." *Proc. helminth. Soc. Wash.*, **27**, 145–150. (W.L. 16747a)

Sandars, D. F., 1960.—"*Hydrophitrema gigantica* n.gen. n.sp. (Trematoda, Digenea) from *Hydrophis elegans* (Gray, 1842) from Australia." *Sobretiro del libro Homenaje al Doctor Eduardo Caballero y Caballero. Mexico D. F.*, 263–268.

Yamaguti, S., 1958.—Systema Helminthum. Volume 1. The Digenetic Trematodes of Vertebrates. Interscience Publishers Inc., New York and London.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 309–314.

The Quantitative Use of the Seinhorst 'Mistifier' to Recover Nematodes from Soil, Faeces and Herbage

By R. F. Sturrock, B.Sc., A.R.C.S.

Research Scholar of the Animal Health Trust, Imperial College, London

During the development of a bioassay technique for use with free-living larval stages of nematodes parasitic in sheep, a number of difficulties were encountered with the standard Baermann funnel extraction of nematodes from faeces and soil. An attempt was made to overcome these difficulties by using the mistifier method described for plant nematodes by Seinhorst (1950) and Goodey (1957), in which the infested material, placed on a sieve over a funnel, is subjected to a fine mist of water from an atomising nozzle. A modification of the Seinhorst apparatus is shown in Fig. 1 where the water containing the nematodes passes down the funnel into a shallow tray. The tray is divided by a weir into two chambers, the bottoms of which are sloped to the centre where a bung facilitates the separate sampling of each chamber. Excess water flows to waste over a second weir leaving the nematodes in a small volume of water, allowing them to be counted by standard techniques.

Experiments

The following experiments were designed to determine the rate of recovery of nematodes, the percentage recovery from a known number of nematodes and the variation in recovery between a replicate series of nozzles.

Faeces from a lamb infected with *Trichostrongylus axei*, cultured for a minimum of seven days at 27°C. to give large numbers of larvae, were used as experimental material throughout the following tests unless otherwise stated.

To determine the rate at which nematodes were recovered, three samples of faeces of about 500g. were mistified for four days giving the following larval recoveries: Sample A=58,000, Sample B=42,000 and Sample C=54,000. The numbers of nematodes, expressed

as percentages of the total recovery after four days, recovered at various time intervals are shown in Table I. The recovery from all three samples was of the same order for each time interval, showing that 90% of the total recovery had taken place after twenty fours ; even after six hours a fairly good recovery (70-80%) had been made.

TABLE I
% of total recovery

Samples	Time on mistifier in hrs.									
	1	2	3	4	5	6	24	48	72	96
A	—	—	—	—	—	72·1	93·6	97·4	99·3	100
B	—	—	—	—	—	80·6	92·8	97·0	99·3	100
C	50·5	63·7	69·2	71·5	75·2	78·6	89·2	97·2	99·3	100

(Not examined)

TABLE II
Variation between samples after 48 hours

Sample	Vol. of filtrate in ml.	No. of larvae/ml. subsample. 4 replicates	Sum of 4 replicates	Chi-square of each sample	Total Chi-Sq.	No. nematodes/ sample
D	100	122+145+108+130	505	0·9866		12,625
E	,,	107+126+142+120	495	0·2898		12,375
F	,,	109+124+105+123	461	1·0169	5·8050	11,525
G	,,	147+112+131+120	510	1·4903		12,750
H	,,	103+119+122+131	475	0·1380		11·875
I	,,	118+115+112+108	453	1·8834		11,325

TABLE III
Passage of nematodes over weir I.

Sample	Chamber 1	Chamber 2	Total No. of nematodes recovered	% of total in Chamber 2	Mean % in Chamber 2
J	19,600	440	20,040	2·19	
K	29,500	2,700	32,200	8·38	
L	72,000	13,000	85,000	15·29	6·2
M	21,000	830	22,630	3·67	
N	11,000	760	12,860	5·91	
O	20,006	380	21,180	1·79	

The variation between a replicate series of nozzles was tested by "mistifying" six 500g. samples of faeces for 48 hours. As these samples had been drawn from a larger quantity of faeces which had been thoroughly mixed, the number of larvae present in each sample should not vary significantly from the mean. Table II shows that there is no significant difference between the recoveries from the six nozzles.

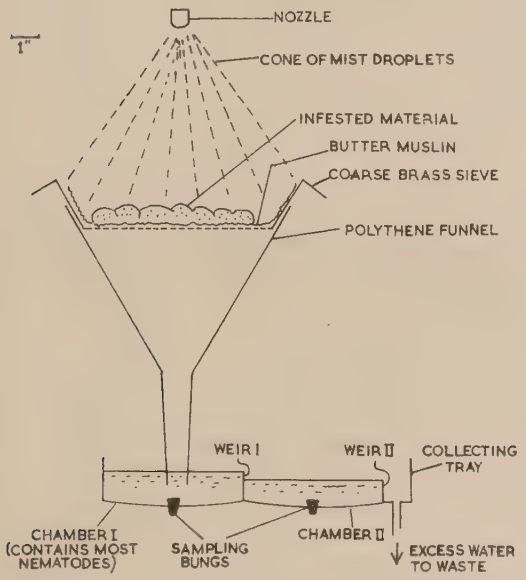


Fig. 1.—Diagram of a single mistifier unit.

The efficiency with which the weirs trapped the nematodes was tested by "mistifying" six 500g. samples of faeces for ninety-six hours. The two chambers of each tray were sampled separately and the proportion of nematodes recovered from the second chamber is shown to be of the order of 6% of the total recovery (Table III).

Since the samples were not drawn from the same source, the recovery from one is not comparable with that of any other. The degree of stirring in individual trays was determined by introducing crystals of potassium permanganate into the trays : it was clearly shown that less stirring occurred in the second chamber than in the first. It was impossible to extract the nematodes from the large quantities of water (2 gal./hr.) which passed over the second weir but since less stirring occurred in the second chamber it is reasonable to suppose that the proportion of nematodes passing over the second weir was no greater than that passing over the first. Hence the overall loss from a tray is probably of the order of 1%.

TABLE IV

Absolute recovery

Sample	Artificial Substrate	No. of nematodes added	No. of Nematodes recovered	% recovery
P			25,130	90·07
Q	Sand A	27,900	25,200	90·32
R			25,800	92·47
S			24,900	89·25
T	Sand B	4,500	4,000	88·88
U			3,500	77·78
V	Cut grass	5,300	4,450	83·97
W			4,400	83·02

To test the absolute recovery, suspensions containing a known number of nematodes previously extracted by the mistifier were placed on clean substrates of grass and sand. After mistifying for 48 hours the recoveries shown in Table IV were obtained. Although the recovery is high (80%), the proportional recovery is less than would be expected from the results of the previous experiment. The difference can probably be explained by the diminished viability of nematodes in the suspension which had already been extracted once by the mistifier method.

Discussion

The mistifier has been used by plant nematologists as a qualitative rather than a quantitative method for recovering nematodes. As originally described by Seinhorst, one nozzle pointing upwards supplied mist for several funnels and in this form the apparatus could not be used quantitatively owing to the uneven distribution of mist droplets. To eliminate such errors from the experiments described in this paper, each nozzle was pointed downwards serving only one funnel.

The results of the above experiments show that the method can be used quantitatively for replicated experiments. It may be argued that in the tests on absolute recovery the nematodes were not under strictly natural conditions. Nevertheless when comparative results are required as opposed to absolute recoveries, the technique is very efficient and is less time-consuming than other methods. It has since been used successfully in this quantitative way both in experiments with trichostrongyle larvae in faecal, soil and grass samples (naturally and artificially infested) and also in experiments with the stem eelworm of narcissus, *Ditylenchus dipsaci*, (J. M. Webster, private communication).

Summary

A modified form of the Seinhorst "Mistifier" is described which allows the quantitative recovery of nematodes from infested material. Experimental results are given showing the high rate of recovery in a short time, the recovery from a known number of nematodes and a not excessive degree of variation between recoveries from a replicated series of mistifier units. These results are discussed and the value of the technique for replicated quantitative work is stressed.

Acknowledgments

I am grateful to Dr. N. G. M. Hague for his advice during the preparation of this paper.

References

Goodey, J. B., 1957.—"Laboratory methods for work with plant and soil nematodes." *Min. Agric. Fish. Fd, Tech. Bull. No.* 2

Seinhorst, J. W., 1950.—"De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* (Kuhn) Filipjev)." *Tijdschr. PlZiekt.*, **56,** 289–348. (W.L. 21280)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, pp. 315–318.

The Passive Transfer of Immunity in Guineapigs Infected with the Nematode *Ascaris suum* Goeze, 1782*

By L. F. Taffs, Ph.D. (Cantab.), M.R.C.V.S.
*Department of Animal Pathology, School of Veterinary Medicine, Cambridge***

Evidence that antibody plays an important part in the protective mechanism of an immune host can be shown by the passive transfer of serum from a resistant animal to a susceptible one, and by subsequently demonstrating that this serum confers on the recipient animal some degree of protection against infection with the homologous parasite. The ability to confer protection by serum inoculation presupposes that an immune response has taken place in the donor animal.

The purpose of this work was to find out if guineapigs could be protected against an oral dose of infective *A. suum* eggs by the intraperitoneal injection of immune anti-*Ascaris* guineapig serum.

Material

Normal guineapig serum

Normal serum was obtained by killing clinically normal guineapigs and collecting blood from the heart and jugular vein. The blood was allowed to clot at room temperature overnight ; the serum was then withdrawn, pooled and stored at –25°C.

Immune guineapig serum

Guineapigs were given orally a number of sublethal doses of *Ascaris* eggs. The animals developed a good acquired immunity and were killed ten or thirteen days after the final reinfection. The antisera were obtained and stored in the same manner as for normal serum.

* Part of a thesis approved by the University of Cambridge for the award of the Ph.D. degree.

** Present address: Department of Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

Experimental Procedure

By intraperitoneal injection three guineapigs (143–145) received immune serum and four (controls 147–150) received normal serum. Each animal was inoculated with a total of 16·5 ml. of serum over a period of six days. The amount of serum each guineapig received per day is shown in Table I.

Table I

Amount of serum each guineapig received intraperitoneally

Days after infection	–1	0	1	2	3	4	5	6	7	8	9
Amount of serum injected per guineapig. (ml.)	4	4	4	2	2	½	–	–	–	–	Killed

All seven guineapigs were infected by mouth with 150,000 *A. suum* eggs on the second day of receiving serum, and were observed later for signs of respiratory distress. Those animals which did not die were killed on the ninth day of infection and a comparison was made of the *post mortem* findings between those animals which received normal serum and those which received immune serum. The degree of haemorrhage in the lung, the number of larvae in the lungs and tracheae and the size of the larvae from the lungs of each guineapig were determined.

Results and Discussion

One control (147), which received normal serum, succumbed to the infection and died on the ninth day. Although this was the only control animal which died within nine days of infection, it is apparent from the results which are presented in Table II that two (143, 144) out of three guineapigs, which were injected with immune serum, received protection from a test dose of eggs. This resistance was manifested (1) by observing before death less marked pneumonic involvement (respiratory rate and dyspnoea) and (2) the findings *post mortem* of less haemorrhage in the lungs, fewer larvae in the lungs and tracheae and the inhibition of growth of the larvae, as compared with the results obtained in the controls which received normal serum. Inhibition of larval development was also seen in the third guineapig (145) which received immune serum.

These results are in general agreement with those of Kerr (1938) and Fallis (1948). Kerr, who used the survival of the host as the criterion of resistance, found that three out of seven guineapigs which received immune serum survived a test infection, while six of the seven controls died. Fallis observed that a slight passive resistance resulted from injections of large quantities of serum from resistant to susceptible guineapigs. This resistance was apparent from the amount of congestion in the lungs and the number and size of the larvae found in the lungs after infection.

Table II

A comparison of signs of pneumonic involvement, degree of lung haemorrhage, number of larvae recovered from the lungs, and measurements of larvae taken from the lungs, in guineapigs which received normal serum and those which received immune serum killed nine days after an oral infection of 150,000 eggs of A. suum.

Guineapig No.	Injected with	Signs of pneumonic involvement	Degree of lung haemorrhage	No. *Ascaris* larvae recovered from		Av. measurements of larvae recovered from the lungs (mm.)	
				Lungs	Trachea		
143	Immune serum	+	+	19	0		Killed 9th day
144	,,	+	+	13	0	1·078 (32)*	Killed 9th day
145	,,	+++	++	700	0	0.939 (50)*	Killed 9th day
147	Normal serum	+++	++	6,600	30		Died 9th day
148	,,	+++	++	1,000	4	1·305 (50)*	Killed 9th day
149	,,	+++	++	900	2		Killed 9th day
150	,,	+++	++	300	3		Killed 9th day

* ( ) Denotes number of larvae measured.

It is possible that by concentrating the antibody in the gamma globulin fraction of the immune serum and passively transferring this that more significant results might be obtained.

SUMMARY

Two out of three guineapigs received some degree of protection against an oral dose of *A. suum* eggs after the intraperitoneal injection of immune anti-*Ascaris* guineapig serum.

ACKNOWLEDGMENTS

This work was done in 1954 under the tenure of an Animal Health Trust Cooper Research Training Scholarship. I am grateful to Dr. E. J. L. Soulsby under whose guidance this investigation was carried out.

REFERENCES

FALLIS, A. M., 1948.—"*Ascaris lumbricoides* infection in guinea-pigs with special reference to eosinophilia and resistance." *Canad. J. Res.* (*Sci.*), **26**, 307–327. (W.L. 5898c)

KERR, K. B., 1938.—"Studies on the passive transference of acquired resistance to the dog hookworm and pig Ascaris." *Amer. J. Hyg.*, **27**, 60–66. (W.L. 600a)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 319–344.

Immunological Studies on Experimental Infection of Pigs with *Ascaris suum* Goeze, 1782

I. An Introduction with a Review of the Literature and the Demonstration of Complement-fixing Antibodies in the Serum*

By L. F. Taffs, Ph.D., M.R.C.V.S.

*Department of Animal Pathology, School of Veterinary Medicine, Cambridge***

Ascaris lumbricoides is probably one of the oldest parasites known to man. It has attracted the attention of workers for many years and of all the species of helminths found in the intestine of man and pig, none perhaps has been more studied. Our knowledge must go back to prehistoric times as it is unlikely that such a cosmopolitan human parasite could have been overlooked. Indeed, the existence of large and small intestinal roundworms, presumably *A. lumbricoides* and *Enterobius vermicularis*, is known to-day among members of certain primitive tribes in South-East Asia (Hoeppli, 1954). It is probable that knowledge of parasites held to-day by primitive races more or less represents the knowledge of their own remote ancestors and of the ancestors of those other races which subsequently became highly civilized (Hoeppli, 1959).

The worm was certainly known in ancient civilizations. It was probably referred to in the translation of the cuneiform texts of Mesopotamia (Hoeppli, 1959) and mentioned in the papyrus Ebers of Egypt, a manuscript which has been dated 1553–1550 B.C. *A. lumbricoides* was also known to early Graeco-Roman physicians who often called it *lumbricus teres*. In the Hippocratic collection (cited by Hoeppli, 1959) it is mentioned as occurring in children.

* Part of a thesis approved by the University of Cambridge for the award of the Ph.D. degree.

** Present address : Department of Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

Aristotle (382–322 B.C.) refers to nematodes, including ascarids, on numerous occasions in his "Historia Animalium" (Chitwood and Chitwood, 1937). In early Chinese medical literature, about 300–200 B.C., it was the first human helminth recorded. It is interesting to note also that in early Chinese medicine *Ascaris* was used as an aphrodisiac, as a remedy for various eye diseases and for the treatment of anal fistulae. (Hoeppli, 1959).

Although this parasite has been known since remote times, it was not until the seventeenth and eighteenth centuries, after the invention of the compound microscope at the end of the sixteenth century, that its anatomy was studied in any detail (Tyson, 1683 ; Redi, 1684 ; Vallisnieri, 1713 ; Linnaeus, 1758). Chitwood and Chitwood (1937) gives 1870 as an approximate date from which recent nematology really begins, since the greater part of our knowledge concerning the life history, anatomy and systematics stems from this time. Certainly, few critical experiments on *Ascaris* were carried out until the end of the nineteenth century. From then onwards our knowledge increased rapidly, particularly during the first half of the twentieth century when the life history was worked out. During this period studies were also made on the nature of the development of the eggs of the worm and the effect of various physical and chemical agents on their viability. It was discovered that infection with this nematode may cause pneumonia, jaundice, intestinal obstruction and enteritis. Investigators also became concerned with the identity and relationship of the human and pig forms of *A. lumbricoides* and the problems of resistance.

Helminthologists have often chosen *A. lumbricoides* for their experiments, because of its large size and the facility with which the somatic part of its life history can take place in laboratory rodents. For these reasons it was considered to be a particularly suitable worm to study in an investigation of immunity to helminth parasites. Although many experiments on immunity have been carried out in rodents, comparatively little immunological work has been done in pigs. The absence of detailed information concerning the mechanism of resistance in the pig was, therefore, the main stimulus for this research. The writer has carried out a variety of experiments both in rodents and pigs and it is hoped to publish these results in a series of papers. Prior to a report of this present work, a note on terminology, an historical review of the life cycle, a discussion on helminth immunity in general and *Ascaris* immunity in the pig in particular, have been included.

Terminology

An examination of the literature on experiments with the human and pig ascarids has shown that some workers have differentiated them taxonomically and some have not. The majority of investigators have called both the human and pig forms *A. lumbricoides* Linnaeus, 1758. However, some earlier workers, although they were unable to detect any morphological differences between the two, thought that because of the difficulty of cross infection between man and pig they were distinct physiologically. Consequently, because of this and also to avoid confusion, Stewart (1916d, 1917a, 1918a) referred to the pig ascarid as *Ascaris suilla* Dujardin, 1845 and the human ascarid as *A. lumbricoides*. Ransom and Foster (1917, 1919, 1920), Ransom (1919) and Payne, Ackert and Hartman (1925) called the pig form *Ascaris suum* Goeze, 1782 or *A. suilla*. Martin (1926) proposed to specify the type of *A. lumbricoides* by the variety names *hominis* and *suis*. More recently, Fenwick (1938, 1939), Kerr (1938b), Pitts (1948), Alicata (1957) and Kelley, Olsen and Hoerlein (1957) have referred to the pig ascarid as *A. suum*. Oliver-González (1943), Chandler (1949), Kagan (1957a, b) and Takata (1951) have used the variety name *A. lumbricoides* var. *suum*.

Certain controversial evidence has been submitted for and against the assumption that the pig and human forms are two distinct worms. Our knowledge has been obtained from the study of their epidemiology, morphology, physiology, serology and toxicology.

For instance, Payne *et al.* (1925), Caldwell and Caldwell (1926) and Roberts (1934) have pointed out that in districts where human and pig *Ascaris* infestations are prevalent, the incidence of infection in man and pig bears no direct relationship.

Bakker (1921), who made a comparative morphological study on the body, cuticular rings, eggs, uteri, spicules and papillae, found no constant differences between the two worms. Further, Barker (1923, cited by Payne *et al.*, 1925) showed that the size, shape and complex of the chromosomes of the eggs of fertile ascarids of man were the same as those described by Boveri, Carnoy, Fürst and Edwards for the pig ascarid. Thornton (1924), who made a morphological comparison between the ascarids of man, swine and chimpanzee, gave further support for this contention, since he could find no essential difference. However, Sprent (1952) has now shown that there is an anatomical difference between the two, based on the nature of the denticles on the lips. This work has

been confirmed by Abdulrachman and Lie Kian Joe (1954). They agree with Sprent's findings that the denticles are specifically distinct, although they found that the distinction is not always sharply defined.

With regard to cross infection, Koino (1922) fed himself with eggs of the pig ascarid and although he suffered from respiratory distress the larvae did not reach maturity. Payne *et al.* (1925) gave themselves eggs from the pig and dosed pigs with eggs of the human ascarid. They were able to cause increased respiration in pigs with eggs of the human ascarid, but they could not produce intestinal infestations in either case. Martin (1926) reported that he was unable to infect pigs with eggs of the human ascarid and thought that morphologically and biologically the worms were indistinguishable. Hiraishi (1928) managed to infect pigs with human *Ascaris* eggs but these animals were kept on a diet deficient in vitamin A. Buckley (1931) fed himself with larvae taken from the lungs of a pig which had died after receiving eggs of the pig ascarid. Control pigs which he had infected with larvae at the same time subsequently became infected, but he was unable to infect himself. On the other hand, de Boer (1935), who remarked that cross infection experiments in the past had suffered from the difficulty of excluding spontaneous infection, fed pigs under strictly controlled conditions with human eggs and later recovered mature egg-producing worms. He concluded that the worms were identical and cross infection can take place. Similar successful results were obtained by Takata (1951). He believes that it is correct to regard the pig strain as a subspecies, *A. lumbricoides* var. *suum*.

In a study of their serology, Schwartz (1920), using the precipitin test in rabbits, found that extracts of *Ascaris* from man were indistinguishable from extracts of *Ascaris* from swine. Further, Bakker (1921) failed to show, both by toxicological and serological studies, any significant difference between the two. However, with the advance of knowledge and the application of new techniques a serological difference has now been shown. In this work Campbell (1937) has indicated that the carbohydrate fractions of each ascarid are immunologically distinct. Also, Kagan (1957a) has demonstrated in rabbits, by selective absorption and haemagglutination inhibition, that antisera prepared against the two ascarid forms can be serologically differentiated from each other.

The work of Sprent and Abdulrachman and Lie Kian Joe on the morphology, the undisputed epidemiological evidence and the serological differences described by Campbell and Kagan suggest that the two worms are distinct. Moreover, it is possible that other

immunological differences will be reported later. One may *perhaps* therefore agree with Cobbold who as long ago as 1864 stated: "This well-known parasite (*A. lumbricoides*) is by some still considered identical with the *Ascaris megalocephala* of the horse, and with the *Ascaris suilla* of the hog; but I have long ago made up my mind that it is a distinct species, in which I am anticipated by Dujardin, Moquin-Tandor, Claparède, and many other helminthologists."

It should not be forgotten, however, that although cross infection is difficult it can occur experimentally (de Boer; Takata). Whether this has a more important epidemiological significance than has been hitherto supposed, it is difficult to say. Evidence obtained so far from epidemiological surveys would suggest that there is little or no cross infection between man and pig in areas where the incidence of either human or pig *Ascaris* infestation is high.

In order to avoid confusion in this and subsequent papers, the pig ascarid will be referred to as *A. suum* and the human form as *A. lumbricoides*.

The Life Cycle

According to Leuckart (1876), Mosler in 1860 produced fever and dyspnoea in children by feeding eggs of *A. lumbricoides*. Davaine (1863) fed rats with infective ova and recovered larvae from the intestines twelve hours later. He believed that hatching and development of *Ascaris* occurred in the alimentary tract. Leuckart (1867, cited by Stewart, 1916a) obtained many negative results in a variety of animals and thought that lower animals might act as intermediary hosts. Such an alternative host, *Julus guttulatus*—a small myriapod—was suggested by von Linstow (1886). The work of Calandruccio (1886, cited by Stewart, 1916a), Grassi (1888), Lutz (1888) and Epstein (1892) however suggested that no intermediate host was involved in the life history. Calandruccio claimed to have successfully infected a child of ten by feeding eggs directly, and Grassi found eggs in his stools after feeding himself with human *Ascaris* eggs. Lutz, having caused fever, bronchitis and diarrhoea by feeding eggs to a volunteer, found immature worms which had been voided following anthelmintic treatment. Epstein infected children with human *Ascaris* eggs and observed eggs in the faeces eighty days later. Wharton (1915) believed that direct infection can take place but that the embryos must be "completely developed".

Stewart (1916a), in a preliminary report of his work, pointed out that the majority of parasitologists considered that the life cycle was simple and direct. It was thought that ingested eggs hatched in the intestine of the host and there developed to adult worms. In this notable contribution, Stewart discovered that not only did *Ascaris* eggs hatch in the intestines of rats and mice, a fact reported by Davaine (1863) half a century earlier, but he was the first to show that some of the liberated larvae entered the body of the host to reach the liver and lungs. He failed to infect pigs with human or pig *Ascaris* eggs. This confirmed many of the negative results obtained by Leuckart and others and Stewart concluded that rats and mice might act as intermediate hosts by the contamination of food and environment with larvae from their saliva or faeces.

In the same month (July, 1916), an Editorial in the *British Medical Journal* supported the view of a direct life cycle, and put forward the hypothesis that *Ascaris* larvae, after hatching in the intestine of the definitive host, reach the liver and lungs and return again to the intestine. Stewart (1916b), who was supported meanwhile by a letter to the Editor from Ross (1916), replied to the Editor and concluded that this hypothesis was untenable because of his and Leuckart's repeated negative results after feeding infective eggs to pigs directly. Later Stewart (1916c, d ; 1917a, b ; 1918a, b) traced the parasite in rats and mice through the liver, lungs and back again to the intestine. He (1917b) was also the first to discover that in pigs the larvae after hatching in the intestine pursued a similar course as far as the trachea. He further managed to infect pigs by feeding lungs of the rat and mouse which had been infected with both human and pig *Ascaris* eggs.

Lane (1917) criticized Stewart's work for various reasons and stated that direct infection had already been proved by the results of previous workers. Stewart (1917c) in reply, pointed out that the successful results obtained by the helminthologists quoted by Lane were not conclusive, because natural infection was prevalent in their districts. Ransom and Foster (1917) supporting Lane, also did not feel justified in accepting Stewart's theory. They thought that Epstein's work on young animals had already proved direct development. They suggested that age may be an important factor and that many of the failures would not have occurred if younger subjects had been used. They confirmed Stewart's work on the migration of larvae in rats and mice, and although they also failed to infect pigs directly, believed that the larvae in pigs migrated through the liver and lungs and then returned to the intestine to complete their development. Low (1918) stated that Stewart's

work had proved the larval vasculo-pulmonary circuit in the pig, but it was unfortunate that some of his pigs had been naturally infected as it rendered it difficult to say whether he reinfected them or not.

Ransom and Foster (1919) showed that *A. suum* could also migrate through the liver and lungs of guineapigs and rabbits and that in goats the larvae developed further than in rodents. They said that the value of Stewart's work lay in the establishment of certain facts relating to the migration of *Ascaris* larvae and not in the suggestion that rodents acted as intermediate hosts. Moreover, they thought that it was unlikely that the larvae could resist unfavourable conditions outside the body.

After further studies, Stewart (1919a, b; 1920; 1921) finally abandoned his idea of the involvement of an intermediate host. Stewart's investigations and those of Yoshida (1919a, b), Ransom and Foster (1920), Fülleborn (1921), Ransom and Cram (1921), Ransom (1922), Martin (1926) and Roberts (1934) not only showed the life cycle to be direct, but also laid the foundation of our knowledge concerning the life history of this nematode parasite.

Immunity to Helminths

It is well known that many animals are able to resist helminth infections. Fundamentally, the defensive mechanisms which are employed by the host against infections with helminth parasites are essentially the same as those forces protecting animals against other infections, since the same humoral and cellular defence systems are used by the host against all types of organisms. However, one feature distinguishes worm infections from bacterial and viral diseases, and that is the inability of helminths to multiply within the body. Bacteria and viruses can propagate themselves by division, whereas helminth numbers can only be increased by successful reinfection. Consequently, it is possible to study the reactions of the host to infection with one single generation of the worm and also to the different developmental stages in that life cycle.

Immunity to helminths, like immunity to any other type of organism, can be divided into natural resistance and acquired resistance. Little detailed knowledge is available about the nature of innate resistance, but the evidence indicates that the degree of natural resistance of the host is largely dependent upon its genetic constitution, diet and age. The majority of parasitologists have concerned themselves with acquired immunity, the prerequisite of which is previous infection. This is often manifested by the ability

to demonstrate in the blood of resistant animals specific antibodies antagonistic to the parasite. By far the greater part of the literature has been related to the study of antigen-antibody reactions using allergic skin tests and the application of known serological techniques, e.g. complement fixation, agglutination and precipitation. The earlier work on immunity was concerned more with diagnosis than with the problems of resistance and has mainly dealt with infestations of man by hydatids, hookworms, schistosomes, *Trichinella spiralis* and *A. lumbricoides*. Pioneers in this field were Ghedini (1906, 1907a, b) who developed a complement fixation test for *Echinococcus*, human ascariasis and hookworm disease, and Casoni (1911) who devised a skin test for *Echinococcus*.

Within the last thirty years much progress has been made in our understanding of immunity to helminths, and ample evidence has now been accumulated to show that both innate and acquired immunity occur in worm infestations. Some manifestations of acquired immunity to nematodes have been demonstrated in infections with : *Haemonchus contortus* (Stoll, 1928, 1929 ; Stewart, 1950a, c, 1953) ; *T. spiralis* (McCoy, 1931 ; Roth, 1939) ; *Nippostrongylus muris* (Africa, 1931 ; Schwartz, Alicata and Lucker, 1931) ; *Trichostrongylus* spp. (Sarles, 1932 ; Taylor, 1934 ; Stewart, 1950b ; Gibson, 1952 ; Michel, 1953 ; Stewart and Gordon, 1953) ; *Dictyocaulus* spp. (Kauzal, 1934 ; Rubin and Lucker, 1956) ; *Metastrongylus elongatus* (Schwartz and Lucker, 1935) ; *Strongyloides* spp. (Sheldon, 1937 ; Turner, 1956), and *A. suum* (in rodents: Wagner, 1933 ; Matsushima, 1934 ; Kerr, 1938a ; Baudet, 1941 ; Fallis, 1942, 1944, 1948 ; Sprent and Chen, 1949).

As Culbertson (1941) points out, resistance can be demonstrated in three main ways :—

(1) *Exposing the animal to reinfection.* One of the best methods of demonstrating immunity is the ability of the host to resist reinfection with the homologous parasite. This may be either complete, which is rare in helminth infections, or partial as in infections with hydatid disease of sheep (Turner, Dennis and Berberian, 1937) and *Ascaridia* infections in chickens (Graham, Ackert and Jones, 1932).

(2) *Applying tests to the serum of the host.* An immune response elicited by the host can be demonstrated by testing the serum *in vitro* using various serological tests for the presence of specific antibodies, or by inducing protection in susceptible animals by the passive transfer of immune serum, as shown by Sarles and Taliaferro (1938) in *N. muris* infection of rats and Otto (1938) in hookworm

disease of dogs. Another indication of protective immunity is the ability of immune serum to cause precipitates to form around larvae. This antigen-antibody reaction was noticed by Sarles (1937, 1938) when larval forms of *N. muris* were immersed in homologous immune rat serum.

Antibodies can be induced in animals by the parenteral administration of fresh or desiccated helminth materials, but usually these antibodies will evoke little or no protective immunity. Generally, it is only when live active worms are allowed to invade the animal that a good immunity is produced.

(3) *Determining the effects of the host upon the parasite.* Although a resistant animal may not prevent the entry of the parasite into the body, the immune status of the host can be detected by the effect it has on the parasite's subsequent residence in that animal. Such effects of immunity on the helminth are : (a) death, (b) inhibition of growth, (c) increases in the time taken to reach maturity, (d) decrease in the production of eggs, and (e) elimination of worms previously established.

This latter phenomenon was designated by Stoll (1929) as "self-cure and protection", since he observed that following reinfection of lambs with *H. contortus*, the egg counts although rising initially to high levels later became negative with the elimination of worms. Thereafter the lambs became refractory to reinfection. Stewart (1953) has shown that this reaction is associated with a marked hypersensitive response in the mucosa of the abomasum and intestine. Of interest in this connection is the phenomenon which occurs in sheep in spring called "spring rise". The phenomenon is manifested at this time by a marked increase in the worm burden and egg output (Morgan and Sloan, 1947 ; Morgan, Parnell and Rayski, 1950, 1951, 1952 ; Crofton, 1954). Soulsby (1957a), indicating that the termination of the "spring rise" has all the characteristics of a "self-cure" mechanism (elevation of antibody titre with reduced egg output and elimination of worms), believes that this phenomenon may be attributed to a lowering of the immune status of the flock, since he noticed that prior to the onset of the "spring rise" a fall of antibody titre occurred.

Retardation of development of helminths in immune animals has been demonstrated in many infections including *Schistosoma japonicum* in dogs (Ozawa, 1930), *Taenia taeniaeformis* in cats (Ohira, 1935) and *N. muris* in rats (Chandler, 1932, 1936a, b, 1937). Chandler (1935a, b, 1953) believes that immunity in *N. muris* infections of rats is due to a reaction against the parasite's metabolic

products which may be enzymes concerned in the utilization of food. He thinks that the resulting phenomena—stunting of growth, failure of development to maturity and inhibition of reproduction — are due to interference with the nutrition of the parasite. This might be designated the anti-enzyme basis of immunity to helminths. It is probable that the metabolic products of worms play an important part in stimulating immunity, as it is well known that immunity induced by disintegrated and dead worms is inferior to that produced by live active forms. Taliaferro (1940a, b) believes that precipitins which are formed by the host are instrumental in affecting the parasite, although more recently (1958) he has pointed out that the antibodies, which are known to be concerned with the prevention of feeding, stunting, immobilisation, occasional death and expulsion of *N. muris* from the intestine following passive transfer, need not necessarily act as precipitins, i.e. be precipitating. Taliaferro states that : "the evidence so far indicates that the mechanisms of immunity rests immediately on humoral factors with secondary cellular co-operation".

Extensive accounts of immunity to parasites have been given by Taliaferro (1929) and Culbertson (1941). More recently, Taliaferro (1940a, b, 1948), Ackert (1942), Lavier (1945), Deschiens and Poirier (1952), Wetzel (1952), Chandler (1953), Shults and Andreeva (1954) and Soulsby (1956, 1957a) have reviewed the problems.

Immunity to *A. suum* in the pig

It is evident from a study of the literature that resistance in the pig to *A. suum* is not well understood. Perhaps the greatest limiting factor which has prevented an assessment and interpretation of results, particularly with regard to age immunity, has been the absence of reliable controlled experimental work due to the extreme difficulty of keeping pigs free from natural infection.

Nevertheless, evidence that resistance to infection exists is suggested by : (1) The difficulty of establishing infections experimentally (Stewart, 1916a ; Ransom and Foster, 1920 ; Martin, 1926 ; Hiraishi, 1927a, b ; Roberts, 1934 ; Clapham, 1936). (2) The decreased frequency of intestinal infestation found in pigs over the age of five months (Ransom and Foster, 1920 ; Roberts, 1934), and (3) the fact that the pathogenic effects of infestation under natural conditions appear to be confined to young pigs (Roberts, 1934).

It is thought that immunity to infection may be due to either a natural and innate resistance, or to the development of an acquired

resistance, or to a combination of both. Resistance may conveniently be discussed under these two headings.

Natural resistance

(a) *The host parasite relationship.* The establishment of experimental intestinal infections in pigs has always been surprisingly difficult compared with the relative success workers have achieved in experimental infections of man, when many worms were often found in the small intestine. In order to account for this difference, Ransom (1922) put forward the hypothesis that man, from an evolutionary standpoint, was the older, better adapted and more essential host, and that the association of the parasite with the pig was more recent and casual. Sandground (1929), in contrasting resistance in the pig with the lack of it in man with their specific ascarids, also believed that *A. suum* was a relatively recently evolved physiological variety of *A. lumbricoides* of man. Similarly, Roberts (1934) thought that the difficulty of infecting pigs might be partly due to an imperfectively adjusted host parasite relationship in which the parasite had not yet reached perfect adjustment with the pig. He believed that his failure to infect pigs was not due to factors preventing larval migration, but rather to the existence of unfavourable conditions in the small intestine for the maturation of the larvae.

(b) *Nutrition.* Roberts (1934) thought that resistance in suckling pigs might be attributed to the milk diet, in that it created either an unfavourable medium in the small intestine for the existence of the larvae, or that the high content of vitamin A in milk might be related in some way with resistance. In support of this latter view he cited the work of Hiraishi (1926 ; 1927a, b), who successfully infected pigs which were on a diet deficient in vitamin A. Prior to this, Hiraishi had infected twenty young pigs which were on a normal diet with only one positive result. It is pertinent to note that Clapham (1934), in an attempt to repeat Hiraishi's work, obtained inconclusive results. In the more recent work of Kelley, Olsen and Hoerlein (1958), the larvae of *A. suum* became established more readily in the intestines of pigs which were on diets of rolled oats, than in pigs which were on diets of milk.

(c) *Age resistance.* Ransom and Foster (1920) showed that the highest percentage of intestinal infestations occurred in pigs between the ages of two and a half months and five months. They believed the lessened susceptibility of pigs over five months of age was due to age rather than to an acquired immunity. Martin (1926) stated that :

"It is apparent that the reason why swine become resistant to *Ascaris* infection is not definitely known, but from the results it appears that this resistance is due to an age factor and not to an immunity". Sandground (1929) pointed out that no definite conclusions could be drawn from previous work because of the very nature of the conditions under which it was practicable to carry out infection experiments. He was more inclined to believe in the existence of an age immunity. Both Morgan (1931) and Clapham (1936) considered that the evidence in favour of an age immunity was far from conclusive. Clapham (1936) claimed to have infected pigs at the age of one year.

From these results there is no evidence to show that the pig develops an age immunity to larval migration, since both Morgan and Clapham were able to obtain successful larval migrations in pigs over five months old.

Roberts (1934) considered that an age immunity might exist, either against the larvae preventing them from establishing themselves in the intestine, or against the adult worms resulting in time in the reduction and expulsion of the worm burden.

Acquired resistance

Stewart (1916c) believed that pigs develop an acquired resistance, since he noticed that in the majority of intestinal infestations the worms were all approximately the same size. Akagi (1931) reported that immunity to *Ascaris* infection had been produced in pigs. Roberts (1934), who also believed that pigs become resistant through infection, thought that this immunity was concerned with the blood leucocytes, particularly the eosinophils, and also with the cells of the reticulo-endothelial system. On the other hand, both Morgan (1931) and Clapham (1936) considered that the evidence in favour of an acquired immunity, as with age immunity, was far from conclusive.

An Outline of the Problem

The main object of this work was to find out if pigs develop an acquired immunity to infection and to see whether age is an important factor in resistance. Since the production of antibodies is often associated with resistance it was hoped : (1) to demonstrate specific antibodies in the serum ; (2) to study the pattern and significance of this antibody response ; and (3) to find out if there was any relationship between protection and degree of immune response.

We know from the work of Soulsby (1957b) that pigs produce antibodies against *Ascaris*. He used the precipitin and intradermal tests and found no correlation between the presence of positive reactions and adult worms in the intestine. However, as far as the writer is aware, little or no other serological work has been carried out in pigs ; certainly, the nature of the response is yet unknown. This is in contrast to the more extensive earlier work carried out on *A. lumbricoides* in man. For instance, Ghedini (1907b), Usami and Kamada (1921) and Isbecque (1924) detected specific antibodies in the serum using the complement fixation test, and precipitins were detected by Coventry and Taliaferro (1928). The phenomenon of hypersensitivity to *Ascaris* in man is also well-known (Ransom, Harrison and Couch, 1924 ; Fülleborn, 1926 ; Coventry and Taliaferro, 1928).

In an earlier study by the writer on the antibody response to infection in rabbits, it was found that antibodies could be detected by the use of the complement fixation test. It was hoped to apply this test directly for the demonstration of antibodies infected in swine. This, however, was not possible because of the procomplementary effect of normal pig serum to guineapig complement. This procomplementary activity of pig serum has been observed by a number of workers who use the complement fixation test in a variety of swine diseases without much success. It was not surprising therefore, as Soulsby (1957b) has pointed out, that Busson (1911) using the standard complement fixation test was unable to detect antibodies in the serum of pigs infected with *A. suum*.

In the majority of sera from normal and immune pigs, heat stable substances are present which augment the haemolytic activity of guineapig complement and which when added to various antigen-antibody mixtures increase the degree of haemolysis. Although the exact nature of the substances responsible for this haemolysis-enhancing activity is not known, it has been suggested by Bankowski, Wichmann and Kummer (1953) that this is due to the third component (C'3) of complement. It has been shown in fractionation studies on sera that the C'3 component is present in much larger amounts in pig sera than in sera of other species (Hegedus and Greiner, 1938 ; Jonsen, Manski and Heidelberger, 1951).

Various attempts have been made to remove the procomplementary substances from normal and immune sera so that the complement fixation test can be applied to the study of swine diseases. Brooksby (1954) was able to use this test in swine exanthema after removing the procomplementary activity of the serum by treatment with liquoid (sodium polyanthole-sulphate),

an anti-coagulant. Rice and McKercher (1954) found that the procomplementary effect could be reduced, but not eliminated, by absorption with sheep red blood cells which removed their high content of sheep red blood cell antibodies. They also considered that the heat stable procomplementary effect of pig serum might be attributed not only to the third component of complement (C'3) and sheep cell antibodies, but also to other constituents such as lipids. They achieved moderate success by treating the pig serum with zymosan which partially removed this haemolysis-enhancing effect. Since Browning (1931) and Petermann (1946) had suggested that the third component (C'3) was related to lipoprotein, Boulanger (1954–55) used three methods in an attempt to remove the procomplementary effect : (1) alcohol fractionation, (2) ether extraction, and (3) *p*H adjustment of the serum. He believed that this last method was the most promising from the practical standpoint of the development of a suitable complement fixation test for the detection of antibodies in swine antisera, so it was the method used in this investigation. The procomplementary substances were precipitated by lowering the *p*H of each sample of serum with hydrochloric acid. In subsequent experiments using the conglutinating complement absorption test, which was found to be more sensitive and suitable, pig serum was treated by the method suggested by Wiggin (1955). These results will be reported later.

The difficulty of keeping pigs free from natural infection was another obstacle which had to be overcome. In later experiments this was partly achieved by obtaining pigs by hysterectomy, the disinfection of pens and the strict isolation of pigs. Unfortunately, it was not until the third year of this investigation that these "colostrum-deprived disease-free" pigs were made available.

The possibility of pre-natal infection of pigs with *Ascaris* had not been overlooked, but under the conditions which were present it was not possible to keep pregnant sows in an "*Ascaris*-free" environment. In any case there is no record of pre-natal infection ever having occurred in pigs. Martin (1926) who investigated this problem stated that : "from the data presented it seems evident that intrauterine infection in swine is very uncommon, and if this phenomenon occurs at all in nature, it must be looked upon as being nothing more than a biological curiosity." Wall (1958) and the writer (unpublished) have also been unsuccessful in demonstrating pre-natal infection in pigs with *Ascaris*.

This paper only records the results of the first experiment. It shows that pigs produce antibodies against *Ascaris* and that this

antibody can be detected in the circulation by means of the complement fixation test after lowering the *p*H of the serum by the addition of hydrochloric acid.

Material and Methods

(a) *Infection of pigs*

Two littermate pigs (1,2) aged nine weeks were available for the experiment. An examination of the faeces showed that one of them (1) had picked up a natural infection. Both pigs were dosed orally with 500,000 *Ascaris* eggs and were killed 124 days later. After infection the pigs were observed for signs of pneumonia and fluctuations of temperature. Faecal samples were collected, and each pig bled, before and after infection. The faeces were examined for *Ascaris* eggs (AEX method, Loughlin and Stoll, 1946) and the sera were tested for antibody against *Ascaris* by means of the complement fixation test.

(b) *Treatment of serum*

Prior to carrying out complement fixation tests, the sera were treated with hydrochloric acid as recommended by Boulanger (1954, 1955). The *p*H of each sample of serum was reduced to *p*H 4·4 by the addition of N/1 hydrochloric acid. The sera were then allowed to stand for 18 hours at 9°C., after which they were neutralized with N/1 sodium hydroxide and tested for complement-fixing antibodies in the standard way. A preliminary titration showed that after treatment, antibody could be detected in two samples of serum which had been obtained after infection, whereas no fixation of complement was observed in the same untreated antisera.

(c) *The complement fixation test*

Tests were carried out on a 50% end point basis using a serum dilution method. Two and a half minimum haemolytic doses of guinea pig complement were employed and the indicator system was made up of 3% sheep red cells and 5 units of haemolysin. Antigen was prepared according to the method described by Blackie (1931) for *Ascaris megalocephala*, slightly modified as follows : Ovaries, oviducts and uteri were dissected out from the adult female *Ascaris* worm and freeze dried for three days. The dried *Ascaris* material was then pulverised in a mortar and stored at 4°C. An alcoholic extract of the powder was prepared by the addition of two grammes of *Ascaris*

material to 100ml. of absolute alcohol. The mixture was incubated at 37°C. for three days and was shaken frequently. It was filtered through a No. 42 filter paper and stored at 4°C. A 1/70 dilution of antigen was used in the test. Antigen-antibody-complement system was incubated in a water-bath at 37°C. and results read. Known positive and negative sera controls were included in each test, and no anticomplementary activity was encountered in the antigen and sera controls which were set up in the usual way.

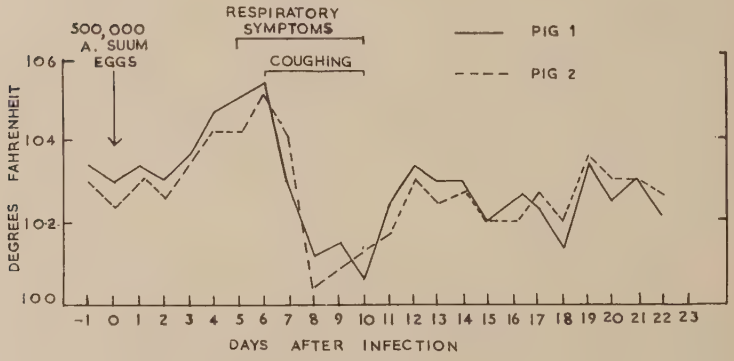


Fig. 1.—Temperature graph of pigs experimentally infected with the eggs of *A. suum*.

Results and Discussion

In both pigs, a rise in temperature from the fourth to sixth days of infection was followed by a subnormal temperature between the eighth and tenth days (Fig. 1). An increase in the respiratory rate of both pigs was observed from the fifth day of infection and by the sixth day the rate was over a hundred a minute; this was accompanied by inappetance. On the sixth day, one pig (1) developed a cough which persisted until the tenth day. Dyspnoea was noticed in both pigs on the sixth, seventh and eighth days. By the tenth day both pigs were eating again and by the twelfth day they appeared clinically normal. These findings are in general agreement with the results of previous workers.

The antibody response and relationship between serum titre and faecal egg count is shown in Fig. 2. *Ascaris* antibody was first detected in the serum of both pigs ten days after infection. Maximum antibody concentration was obtained between the thirteenth and seventeenth days ; thereafter there was a decline in the antibody content of the serum to the thirty-fourth day in pig (1) and to the forty-first day in pig (2). After this period no antibody could be detected in either pig.

While these results can in no way be regarded as the primary antibody response, since one pig (1) had already been naturally infected before experimental infection, it is interesting to note that similar observations, which will be reported later, were obtained in three "colostrum-deprived disease-free" pigs which had been reared free from natural *Ascaris* infection after hysterectomy. In this work, by means of the conglutinating complement absorption test using a saline extract of whole worm as antigen, antibody was also first detected on the tenth day of infection. The peak of serum antibody concentrations was reached on the seventeenth day in two pigs and by the fifty-fifth day in the third. Using this technique it was possible to detect antibodies for a longer period, i.e. for at least as long as 97 days post infection, than with the complement fixation test. Recent work has shown that by feeding a small number of eggs each day to pigs, serum antibodies can be demonstrated for at least as long as six months, as well as conferring a high degree of resistance to reinfection.

In pig 1 an inverse relationship between serum titre and faecal egg count was observed. This inverse relationship, similar to that described by Stewart (1950a, b, c) in his studies of helminthiasis in sheep, was also noticed in later experiments and will be discussed then. Forty-nine days after infection the egg count of this pig (1) became negative.

The infection did not become patent in either pig. Eggs which were observed in the faeces between the third and seventh days of infection were eggs passing through the intestine from the infective dose. Experience has shown that it is easier to infect pigs if smaller does are given, either as a single dose, or by feeding a small number of eggs each day over a period of time, thus simulating natural conditions.

At *post mortem* examination the lungs of both pigs appeared macroscopically normal. The livers were cirrhotic and showed a number of "milk spot" or "white spot" lesions. Two small nodules, a few millimetres in diameter, similar to those described by White (1941), were found beneath the liver capsule of pig 1. No mature

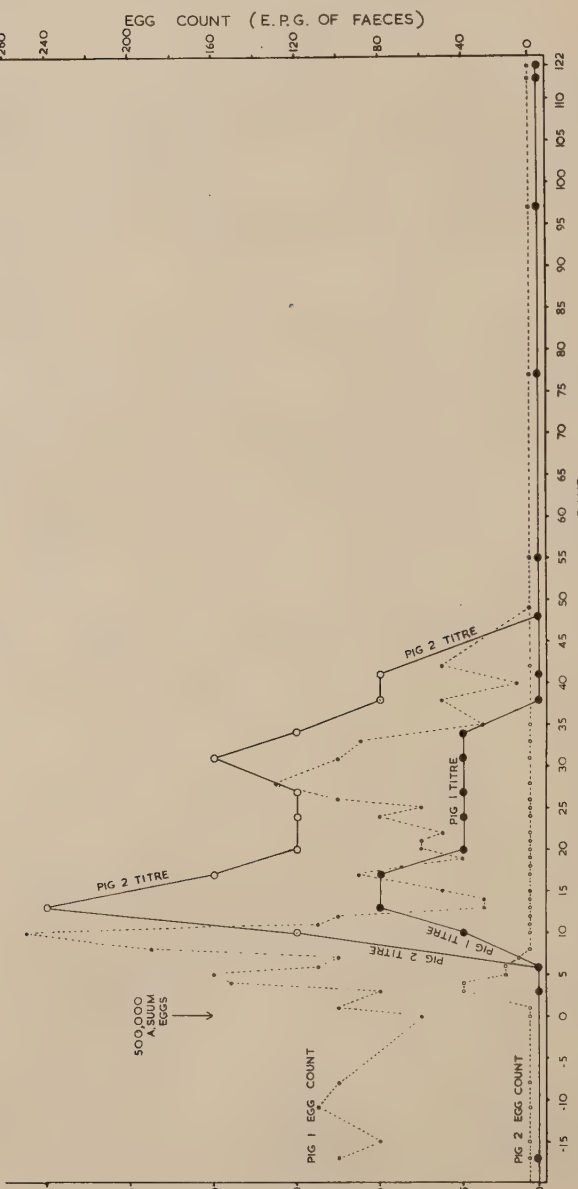


Fig. 2.—The complement fixation test in pigs experimentally infected with the eggs of *A. suum*.

worms were found in the small intestine of either pig. The histopathology of ascariasis will be described in another paper.

SUMMARY

1. An introduction to a study of immunity against *A. suum* in the pig is given. A review of the literature on the life cycle, a note on terminology and a general discussion on immunity to helminths has also been included.

2. By first removing the procomplementary activity of pig serum to guineapig complement with hydrochloric acid, it was possible to apply the complement fixation test to a study of the antibody response to experimental infection with *A. suum*.

3. Using an alcoholic extract of uterus, oviducts and ovaries of the adult female *Ascaris* worm as antigen, antibodies against this nematode were first detected in two experimentally infected pigs ten days after infection. A peak of antibody concentration from the thirteenth to the seventeenth day of infection was followed by a decline in the antibody content of the serum to the forty-first day; after which no antibody was detected.

ACKNOWLEDGMENTS

This investigation was carried out in 1955 in the Department of Animal Pathology, Milton Road, Cambridge. I am grateful to the Animal Health Trust for the award of a Cooper Research Training Scholarship and to Dr. E. J. L. Soulsby for supervising the work.

REFERENCES

ABDULRACHMAN, S. and LIE KIAN JOE, 1954.—"Morphological differences between *Ascaris* from man and pigs." *Docum. Med. geogr. trop. Amst.*, **6**, 342–344.

ACKERT, J. E., 1942.—"Natural resistance to helminthic infections." *J. Parasit.*, **28**, 1–24. (W.L. 11428)

AFRICA, C. M., 1931.—"Studies on the host relations of *Nippostrongylus muris*, with special reference to age resistance and acquired immunity." *Ibid.*, **18**, 1–13.

AKAGI, K., 1931.—"Experimental studies on the immunity of *Ascaris*." Ni. Ikwa. Daigaku *Z.*, **2**, 488-499. *Abstr. in Jap. J. Zool.*, 1933, **4**, 105. (W.L. 1088w)

ALICATA, J. E., 1957.—"Observations on the ineffectiveness of 'polybor-3' in destroying the vitality of the eggs of *Ascaris suum* in soil." *J. Parasit.*, **43**, 193. (W.L. 11428)

BAKKER, C. R., 1921.—"Over de Identiteit van *Ascaris lumbricoides* en *Ascaris suilla*." *Tijdschr. vergelijk. Geneesk.*, **6**, 160–230. (W.L. 21288)

BANKOWSKI, R. A., WICHMANN, R. and KUMMER, M., 1953.—"A complement-fixation test for identification and differentiation of immunological types of the virus of vesicular exanthema of swine." *Amer. J. vet. Res.*, **14**, 145–149. (W.L. 628a)

BARKER, F. D., 1923.—"The chromosomes in *Ascarus* (*Ascaris*) *lumbricoides* of man." (*Abstr.*) *Anat. Rec.*, **24,** 375. (W.L. 763)

BAUDET, E. A. R. F., 1941.—"Über die Resistenz des Meerschweinchens gegenüber weiderholten Infektionen mit *Ascaris lumbricoides* vom Schwein." *Z. InfektKr. Haustiere,* **58,** 79–93. (W.L. 23460)

BLACKIE, W. K., 1931.—"Observations on the immunity in experimental ascariasis in rabbits." *J. Helminth.*, **9,** 91–96. (W.L. 11224c)

DEBOER, E., 1935.—"Experimenteel onderzoek betreffende *Ascaris lumbricoides* van mensch en varken." *Tijdschr. Diergeneesk.*, **62,** 965–973. (W.L. 21262)

BOULANGER, P., 1954.—"A preliminary note on a method of inactivating a substance or substances in swine serum that interfere with the detection of antibodies by the complement-fixation test." *Canad. J. comp. Med.*, **18,** 423–425. (W.L. 5897b)

————, 1955.—"Complement-fixation tests of swine serum. I. In the diagnosis of vesicular stomatitis." *Ibid.*, **19,** 37–47.

BROOKSBY, J. B., 1954.—"Etude expérimentale de l'exanthème vésiculeux." *Bull. Off. int. Epiz.*, **42,** 368–377. (W.L. 4683d)

BROWNING, C. H., 1931.—A system of bacteriology in relation to medicine. **6,** *Immunity,* 332–352. *Medical Research Council, H.M.S.O., Lond.*

BUCKLEY, J. J. C., 1931.—"An observation on human resistance to infection with *Ascaris* from the pig." *J. Helminth.*, **9,** 45–46. (W.L. 11224c)

BUSSON, B., 1911.—"Der Parasitennachweis mittels der Komplementablenkungsmethode." *Zbl. Bakt.* (Orig.), **60,** 426–433. (W.L. 23684)

CALDWELL, F. C. and CALDWELL, E. L., 1926.—"Are *Ascaris lumbricoides* and *Ascaris suilla* identical?" *J. Parasit.*, **13,** 141–145. (W.L. 11428)

CAMBELL, D. H., 1937.—"The immunological specificity of a polysaccharide fraction from some common parasitic helminths." *Ibid.*, **23,** 348–353.

CASONI, T., 1911.—"La diagnosi biologica dell'echinococcosi umana mediante l'intradermoreazione." *Folia clin. chim. micr., Bologna,* **4,** 5–16. (W.L. 8542g)

CHANDLER, A. C., 1932.—"Experiments on resistance of rats to superinfection with the nematode *Nippostrongylus muris*." *Amer. J. Hyg.*, **16,** 750–782. (W.L. 600a)

————, 1935a.—"Studies on the nature of immunity to intestinal helminths. I. The local nature of the immunity of white rats to *Nippostrongylus* infection." *Ibid.*, **22,** 157–168.

————, 1935b.—"Studies on the nature of immunity to intestinal infections. II. A study of the correlation between degree of resistance of white rats to *Nippostrongylus* and interval between infections." *Ibid.*, **22,** 243–256.

————, 1936a.—"Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in *Nippostrongylus* after transfer from immune to non-immune rats." *Ibid.*, **23,** 46–54.

————, 1936b.—"Studies on the nature of immunity to intestinal helminths. IV. The interrelations between parenteral and intestinal immunity in rats infected with *Nippostrongylus*." *Ibid.*, **24,** 129–144.

————, 1937.—"Studies on the nature of immunity to intestinal helminths. V. Experiments on the rôle of the skin in parenteral immunity, and further experiments on passive immunization in *Nippostrongylus* infection in rats." *Ibid.*, **26,** 292–308.

————, 1949. Introduction to parasitology with special reference to the parasites of man. p. 422, Eighth Edition, Chapman and Hall Ltd. Lond.

————, 1953.—"Immunity in parasitic diseases." *J. Egypt. med. Ass.*, **36,** 811–834. (W.L. 11172a)

CHITWOOD, B. G. and CHITWOOD, M. B., 1937.—An introduction to nematology. Monumental Printing Co., Baltimore, U.S.A.

CLAPHAM, P. A., 1934.—"Ascariasis and vitamin A deficiency in pigs." *J. Helminth.*, **12,** 165–176. (W.L. 11224c)

————, 1936.—"Preliminary observations on the infectivity of *Ascaris lumbricoides* to swine." *Ibid.*, **14,** 229–232.

COBBOLD, T. S., 1864. Entozoa : An introduction to the study of helminthology with reference, more particularly, to the internal parasites of man, p. 302, Groombridge & Sons, London.

COVENTRY, F. A. and TALIAFERRO, W. H., 1928.—"Hypersensitiveness to helminth proteins. I. Cutaneous tests with proteins of *Ascaris*, hookworm and *Trichuris* in Honduras." *J. prev. Med., Baltimore,* **2,** 273–288. (W.L. 11467a)

CROFTON, H. D., 1954.—"Nematode parasite populations in sheep on lowland farms. I. Worm egg counts in ewes." *Parasitology,* **44,** 465–477. (W.L. 16035)

CULBERTSON, J. T., 1941. Immunity against animal parasites. Columbia, New York.

DAVAINE, C. J., 1863.—"Nouvelles recherches sur le développement et la propagation de l'ascaride lombricoïde et du trichocéphale de l'homme." *C.R. Soc. Biol.*, (*1862*), **4,** mém., 261–265. (W.L. 6630)

DESCHIENS, R. and POIRIER, M., 1952.—"L'immunité dans les infestations parasitaires." *Ann. Inst. Pasteur,* **83,** 725–744. (W.L. 857)

EDITORIAL, 1916. *British med. J.*, **2,** 23. (W.L. 3579)

EPSTEIN, A., 1892.—"Ueber die Uebertrangund des menschlichen Spulwurms (*Ascaris lumbricoides*)." *Verh. Ges. Kinderheilk., Wiesbaden,* (1891), **9,** 1–16. (W.L. 22323a)

FALLIS, A. M., 1942.—"Resistance to *Ascaris lumbricoides* L. in guinea pigs and the eosinophilia associated with infection." *J. Parasit.*, **28,** (Suppl.), 21. (W.L. 11428)

————, 1944.—"Resistance to *Ascaris lumbricoides* L. infection as demonstrated experimentally in guinea pigs." *Canad. J. publ. Hlth.*, **35,** 90. (W.L. 5898b)

————, 1948.—"*Ascaris lumbricoides* infection in guinea pigs with special reference to eosinophilia and resistance." *Canad. J. Res.*, **26,** 307–327. (W.L. 5898c)

FENWICK, D. W., 1938.—"The oxygen consumption of newly-hatched larvae of *Ascaris suum.*" *Proc. zool. Soc. Lond.*, **108,** 85–100. (W.L. 16969b)

————, 1939.—"Studies on the saline requirements of the larvae of *Ascaris suum.*" *J. Helminth.*, **17,** 211–228. (W.L. 11224c)

FÜLLEBORN, F., 1921.—"Über die Wanderung von Askaris-und anderen Nematodenlarven im Körper und intrauterine Askarisinfektion." (Abstr.) *Arch. Schiffs- u. Tropenhyg.*, **25,** 146–149. (W.L. 1804)

————, 1926.—"Demonstration Über Kutanreaktion bei Helminthenaffektionen." *Ibid.*, **30,** 86–88.

GHEDINI, G., 1906.—"Ricerche sul siero di sangue di individuo affetto da cisti da echinococco e sul liquido in essa contenuto." *Gazz. Osp. Clin.*, **27,** 1616–1617. (W.L.8975)

————, 1907a.—"Sugli anticorpi elminthiaci nel siero di sangue di individui affetti da elminthiasi. Anticorpi echinococcici. Seconda nota." *Ibid.*, **28,** 53.

————, 1907b.—"Anticorpi elmintiaci nel siero di sangue di individui affetti da elmintiasi ; anticorpi anchilostomiaci e ascaride. III. nota." *Ibid.*, **28,** 476.

GIBSON, T. E., 1952.—"The development of acquired resistance by sheep to infestation with the nematode *Trichostrongylus axei*." *J. Helminth.*, **26,** 43–53. (W.L. 11224c)

GRAHAM, G. L., ACKERT, J. E. and JONES, R. W., 1932.—"Studies on an acquired resistance of chickens to the nematode *Ascaridia lineata*." *Amer. J. Hyg.*, **15,** 726–740. (W.L. 600a)

GRASSI, B., 1888.—"Weiterers zur Frage der Ascarisentwicklung.". *Zbl. Bakt.*, **3,** 748–749. (W.L. 23684)

HEGEDUS, A. and GREINER, H., 1938.—"Quantitative Bestimmung der Komplementbestandteile." *Z. ImmunForsh.*, **92,** 1–9. (W.L. 23456)

HIRAISHI, T., 1926.—"Experimental ascariasis of the young pigs with special reference to A-avitaminosis." *Keiô Igaku, Tokyo,* **6,** 79–80.

————, 1927a.—"*Ibid.*" (Abstr.) *Japan med. World,* **7,** 80. (W.L. 10880)

————, 1927b.—"Pig infection with the ascarid larvae." (A supplementary report.) (Abstr.) *Ibid.*, **7,** 243–244.

————, 1928.—"Experimentelle Infektion junger Schweine mit Ascariden mit Rücksicht auf die besonderen Beziehungen zu A-Avitaminose." (Abstr.) *Arch. Schiffs-u. Tropenhyg.*, **32,** 519–521. (W.L. 1804)

HOEPPLI, R., 1954.—"Some early views on parasites and parasitic infections showed by the people of Borneo, Malaya and China." *Proc. Alumni Ass., King Edw. VII Coll. Med. Singapore,* **7,** 3–17. (W.L. 16598a)

————, 1959.—Parasites and parasitic infections in early medicine and science. Singapore University of Malaya Press.

ISBECQUE, G., 1924.—"Recherche d'anticorps spécifiques chez les porteurs de vers intestinaux." *C.R. Soc. Biol., Paris,* **90,** 691–692. (W.L. 6630)

JONSEN, J., MANSKI, W. and HEIDELBERGER, M., 1951. "Pig complement and its fractionation for the production of reagents containing third component." *J. Immunol.*, **67,** 385–391. (W.L. 11238)

KAGAN, I. G., 1957a.—"Haemagglutination studies with *Ascaris* antigens." *J. Parasit.*, **43,** (Suppl.) 44. (W.L. 11428)

————, 1957b.—"Serum-agar double diffusion studies with *Ascaris* antigens." *J. infect., Dis.*, **101,** 11–19. (W.L. 11250)

KAUZAL, G., 1934.—"Observations on the development of resistance to *Dictyocaulus filaria*." *Aust. vet. J.*, **10,** 100–111. (W.L. 2254a)

KELLEY, G. W., OLSEN, L. S. and HOERLEIN, A. B., 1957.—"Rate of migration and growth of larval *Ascaris suum* in baby pigs." *Proc. helm. Soc. Wash.*, **24,** 133–136. (W.L.16747a)

————, 1958.—"The influence of diet on the development of *Ascaris suum* in the small intestines of pigs." *Amer. J. vet. Res.*, **19,** 401–404. (W.L. 628a)

KERR, K. B., 1938a.—"The cellular response in acquired resistance in guinea pigs to an infection with pig *Ascaris*." *Amer. J. Hyg.*, **27,** 28–51. (W.L. 600a)

————, 1938b.—"Studies on the passive transference of acquired resistance to the dog hookworm and pig *Ascaris*." *Ibid.*, **27,** 60–66.

KOINO, S., 1922.—"Experimental infection on human body with ascarides." *Japan med. World,* **2,** 317–320. (W.L. 10880)

LANE, C. L., 1917.—"Major Stewart on *Ascaris* infection." *Indian med. Gaz.*, **52,** 301. (W.L. 9943)

LAVIER, G., 1945.—"Les phénomènes d'immunité dans l'éosinophilie des helminthiases." *Bull. Acad. Méd., Paris,* **129,** 560–561. (W.L. 3683)

LEUCKART, R., 1876. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten. Ein Hand-und Lehrbuch für Naturforscher und Aertze, **2,** p. 222. Leipzig u. Heildelberg.

LINNAEUS, C., 1758.—Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. **1,** p. 648, Holmiae.

VON LINSTOW, O. F. B., 1886.—"Über den Zwischenwirth von *Ascaris lumbricoides* L." *Zool. Anz.*, **9,** 525–528. (W.L. 23833)

LOUGHLIN, E. H. and STOLL, N. R., 1946.—"An efficient concentration method (AEX) for detecting helminthic ova in feces." *Amer. J. trop. med.*, **26,** 517–527. (W.L. 626)

LOW, G. C., 1918.—"The life-history of *Ascaris lumbricoides*." *Brit. med. J.*, **1,** 286. (W.L. 3579)

LUTZ, A., 1888.—"Zur Frage der Uebertragung des menschlichen Spulwurms. Weitere Mitteilungen." *Zbl. Bakt.*, **3,** 425–428. (W.L. 23684)

MARTIN, H. M., 1926.—"Studies on the *Ascaris lumbricoides*." *Res. Bull. Neb. agric. Exp. Sta.*, No. 37. (W.L. 18683)

MATSUSHIMA, M., 1934.—"Experimental studies on the resistance against repeated infection of *Ascaris*." *Keiô Igaku, Tokyo,* **14,** 1119–1134.

MCCOY, O. R., 1931.—"Immunity of rats to reinfection with *Trichinella spiralis*." *Amer. J. Hyg.*, **14,** 484–494. (W.L. 600a)

MICHEL, J. F., 1953.—"Phenomenon of protection in infections of *Trichostrongylus retortaeformis*." *Nature, Lond.*, **172,** 312. (W.L. 14900)

MORGAN, D. O., 1931.—"Some observations on experimental ascariasis in pigs." *J. Helminth.*, **9,** 121–128. (W.L. 11224c)

———— and SLOAN, J. E. N., 1947.—"Researches on helminths in hill sheep with special reference to seasonal variations in worm egg output." *Scot. Agric.*, **27,** 28–33. (W.L. 20001f)

————, PARNELL, I. W., and RAYSKI, C., 1950.—"Further observations on the seasonal variation in worm egg outputs in Scottish hill sheep." *J. Helminth.*, **24,** 101–122. (W.L. 11224c)

————, ————, ————, 1951.—"The seasonal variations in the worm burden of Scottish hill sheep." *Ibid.*, **25,** 177–212.

————, ————, ————, 1952.—"Worm infestations in Scottish hill sheep." *Scot. Agric.*, **32,** 46–53. (W.L. 20001f)

OHIRA, T., 1935.—"On the active immunization of animals against tape worms." *Trans. Far-East Ass. trop. Med.*, (*9th Congress*), **1,** 601–604. (W.L. 21489)

OLIVER-GONZÁLEZ, J., 1943.—"Antigenic analysis of the isolated tissues and body fluids of the roundworm, *Ascaris lumbricoides var. suum*." *J. infect. Dis.*, **72,** 202–212. (W.L. 11250)

OTTO, G. F., 1938.—"Studies on the protective power of serum from dogs actively immunized against *Ancylostoma caninum*." *J. Parasit.*, **24,** (Suppl.) 10. (W.L. 11428)

OZAWA, M., 1930.—"Experimental study on acquired immunity to *schistosomiasis japonica*." *Jap. J. exp. Med.*, **8,** 79–84. (W.L. 10881k)

PAYNE, F. K., ACKERT, J. E. and HARTMAN, E., 1925.—"The question of the human and pig *Ascaris*." *Amer. J. Hyg.*, **5,** 90–101. (W.L. 600a)

PETERMANN, M. L., 1946.—"The effect of lecithinase on human serum globulins." *J. biol. Chem.*, **162,** 37–42. (W.L. 11063)

PITTS, T. D., 1948.—"Experimental hatching of the eggs of *Ascaris suum*." *Proc. Soc. exp. Biol.*, **69,** 348–351. (W.L. 16913)

RANSOM, B. H., 1919.—"A newly recognised cause of pulmonary disease—*Ascaris lumbricoides*." *J. Amer. med. Ass.*, **73,** 1210–1212. (W.L. 11006)

————, 1922.—"Some recent additions to the knowledge of ascariasis." *Ibid.*, **79,** 1094–1097.

RANSOM, B. H. and FOSTER, W. D., 1917.—"Life history of *Ascaris lumbricoides* and related forms." *J. agric. Res.*, **11,** 395–398. (W.L. 10966)

————, ————, 1919.—"Recent discoveries concerning the life history of *Ascaris lumbricoides*." *J. Parasit.*, **5,** 395–398. (W.L. 11428)

————, ————, 1920.—"Observations on the life history of *Ascaris lumbricoides*." *Bull. U.S. Dep. Agric.*, No. 817, 1–47. (W.L. 5641)

———— and CRAM, E. B., 1921.—"The course of migration of *Ascaris* larvae." *Amer. J. trop. Med.*, **1,** 129–160. (W.L. 626)

————, HARRISON, W. T. and COUCH, J. F., 1924.—"*Ascaris* sensitization." *J. agric. Res.*, **28,** 577–582. (W.L. 10966)

REDI, F., 1684.—Osservazioni intorno agli animali viventi che si trovano negli animali viventi. Firenze.

RICE, C. E. and MCKERCHER, P. D., 1954.—"Studies of the complement fixation reaction in virus systems. VI. In vesicular stomatitis in horses, cattle, and swine." *J. Immunol.*, **73,** 309–317. (W.L. 11238)

ROBERTS, F. H. S., 1934.—"The large roundworm of pigs, *Ascaris lumbricoides* L., 1758. Its life history in Queensland, economic importance and control." *Bull. Animal Hlth. Sta. Yeerongpilly, Australia.* No. 1.

ROSS, R., 1916.—"The life-history of *Ascaris lumbricoides*." *Brit. med. J.*, **2,** 60–61. (W.L. 3579)

ROTH, H., 1939.—"Experimental studies on the course of *trichina* infection in guinea pigs. III. Immunity of guinea pigs to re-infection with *Trichinella spiralis*." *Amer. J. Hyg.*, **30,** 35–64. (W.L. 600a)

RUBIN, R. and LUCKER, J. T., 1956.—"Acquired resistance to *Dictyocaulus viviparus*, the lungworm of cattle." *Cornell Vet.*, **46,** 88–96. (W.L. 6888)

SANDGROUND, J. H., 1929.—"A consideration of the relation of host-specificity of helminths and other metazoan parasites to the phenomenon of age resistance and acquired immunity." *Parasitology*, **21,** 227–255. (W.L. 16035)

SARLES, M. P., 1932.—"Development of an acquired resistance in rabbits by repeated infection with an intestinal nematode, *Trichostrongylus calcaratus* Ransom, 1911." *J. Parasit.*, **19,** 61–82. (W.L. 11428)

————, 1937.—"The in *vitro* action of immune rat serum on *Nippostrongylus muris* (Nematoda)." *Ibid.*, **23,** 560–561.

————, 1938.—"The *in vitro* action of immune rat serum on the nematode, *Nippostrongylus muris*." *J. infect. Dis.*, **62,** 337–348. (W.L. 11250)

———— and TALIAFERRO, W. H., 1938.—"The effect of dosage and interval after infection on passive immunity to the nematode, *Nippostrongylus muris*." *J. Parasit.*, (*Suppl.*) **24,** 35. (W.L. 11428)

SCHWARTZ, B., 1920.—"The biological relationships of ascarids." *Ibid.*, **6,** 115–123.

————, ALICATA, J. E. and LUCKER, J. T., 1931.—"Resistance of rats to superinfections with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes." *J. Wash. Acad. Sci.*, **21,** 259–261. (W.L. 11600)

————and LUCKER, J. T., 1935.—"Experimental infections and superinfections of pigs with lungworms." *J. Parasit.*, **21,** 432. (W.L. 11428)

SHELDON, A. J., 1937.—"Studies on active acquired resistance, natural and artificial, in the rat to infection with *Strongyloides ratti*." *Amer. J. Hyg.*, **25,** 53–65. (W.L. 600a)

SHULTS, R. S. and ANDREEVA, N. K., 1954.—[Some rules of immunity to helminths.] *Trud. Inst. Vet., Alma-Ata*, **6,** 468–491. [In Russian.]

SOULSBY, E. J. L., 1956.—"Immunity to helminths." *Vet. Excerpts, Lond.* 10–15. (W.L. 22517f)

SOULSBY, E. J. L., 1957a.—"Some immunological phenomena in parasitic infections." *Vet. Rec.*, **69,** 1129–1136. (W.L. 22523)

————, 1957b.—"Precipitin and intradermal tests in *Ascaris lumbricoides* infection in pigs." *Brit. vet. J.*, **113,** 492–498. (W.L. 3589l)

SPRENT, J. F. A., 1952.—"Anatomical distinction between human and pig strains of *Ascaris*." *Nature, Lond.*, **170,** 627–628. (W.L. 14900)

———— and CHEN, H. H., 1949.—"Immunological studies in mice infected with the larvae of *Ascaris lumbricoides*. I. Criteria of immunity and immunizing effect of isolated worm tissues." *J. infect. Dis.*, **84,** 111–124. (W.L. 11250)

STEWART, D. F., 1950a.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. II. The antibody response to infestation with *H. contortus*." *Aust. J. agric. Res.*, **1,** 301–321. (W.L. 2244a)

————, 1950b.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. III. The antibody response to infestation with *Trichostrongylus* spp." *Ibid.*, **1,** 413–426.

————, 1950c.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. IV. The antibody response to natural infestation in grazing sheep and the 'self-cure' phenomenon." *Ibid.*, **1,** 427–439.

————, 1953.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. V. The nature of the 'self-cure' phenomenon." *Ibid.*, **4,** 100–117.

———— and GORDON, H. McL., 1953.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. VI. The influence of age and nutrition on resistance to *Trichostrongylus colubriformis*." *Ibid.*, **4,** 340–348.

STEWART, F. H., 1916a.—"On the life-history of *Ascaris lumbricoides*." *Brit. med. J.*, **2,** 5–7. (W.L. 3579)

————, 1916b.—"The life-history of *Ascaris lumbricoides*." *Ibid.*, **2,** 474.

————, 1916c.—"Further experiments on *Ascaris* infection." *Ibid.*, **2,** 486–488.

————, 1916d.—"On the life-history of *Ascaris lumbricoides*." *Ibid.*, **2,** 753–754.

————, 1917a.—"On the development of *Ascaris lumbricoides* Lin. and *Ascaris suilla* Duj. in the rat and mouse." *Parasitology*, **9,** 213–227. (W.L. 16035)

————, 1917b.—"Note on *Ascaris* infection in man, the pig, rat, and mouse." *Indian med. Gaz.*, **52,** 272–273. (W.L. 9943)

————, 1917c.—"The life-history of *Ascaris lumbricoides*." *Ibid.*, **52,** 379–380.

————, 1918a—"On the development of *Ascaris lumbricoides* and *A. mystax* in the mouse." *Parasitology*, **10,** 189–196. (W.L. 16035)

————, 1918b.—"On the life history of *Ascaris lumbricoides* L." *Ibid.*, **10,** 197–205.

————, 1919a.—"Recent experiments on the life-history of *Ascaris lumbricoides*." *Brit. med. J.*, **1,** 102. (W.L. 3579)

————, 1919b.—"On the life history of *Ascaris lumbricoides*, L." *Parasitology*, **11,** 385–387. (W.L. 16035)

————, 1920.—"Life-history of *Ascaris lumbricoides*." *Brit. med. J.*, **2,** 818–819. (W.L. 3579)

————, 1921.—"On the life history of *Ascaris lumbricoides*, L. Part V." *Parasitology*, **13,** 37–47. (W.L. 16035)

STOLL, N. R., 1928.—"The occurrence of self-cure and protection in typical nematode parasitism." *J. Parasit.*, **15,** 147–148. (W.L. 11428)

————, 1929.—"Studies with the strongyloid nematode, *Haemonchus contortus*." *Amer. J. Hyg.*, **10,** 384–418. (W.L. 600a)

TAKATA, I., 1951.—"Experimental infection of man with *Ascaris* of man and the pig." *Kitasato Arch.*, **23,** 49–59. (W.L. 11796)

TALIAFERRO, W. H., 1929. The immunology of parasitic infections. Century, New York.

————, 1940a.—"The mechanism of immunity to metazoan parasites." *Amer. J. trop. Med.*, **20,** 169–182. (W.L. 626)

————, 1940b.—"The mechanism of acquired immunity in infections with parasitic worms." *Physiol. Rev.*, **20,** 469–492. (W.L. 16263)

————, 1948.—"The inhibition of reproduction of parasites by immune factors." *Bact. Rev.*, **12,** 1–17. (W.L. 2364a)

————, 1958.—"The synthesis and activities of antibodies." *Rice Inst. Pamphl.*, **45,** 114–140. (W.L. 19349)

TAYLOR, E. L., 1934.—"Field experiments on the immunity of lambs to parasitic gastritis caused by a mixed infection of trichostrongylid nematodes." *J. Helminth.*, **12,** 143–164. (W.L. 11224c)

THORNTON, H., 1924.—"The relationship between the ascarids of man, pig and chimpanzee." *Ann. trop. Med. Parasit.*, **18,** 99–100. (W.L. 1063)

TURNER, E. L., DENNIS, E. W. and BERBERIAN, D. A., 1937.—"The production of artificial immunity against hydatid disease in sheep." *J. Parasit.*, **23,** 43–61. (W.L. 11428)

TURNER, J. H., 1956.—"Further studies on strongyloidiasis in lambs and kids: development of acquired resistance." *Ibid.*, **42,** (Suppl.) 18–19.

TYSON, E., 1683.—"Lumbricus teres, or some anatomical observations on the round worm bred in human bodies." *Phil. Trans.*, **13,** 154–161. (W.L. 16192)

USAMI, K. and KAMADA, R., 1921.—"Complement fixation test in ascariasis." *Chuo Igakkwai Z. Nagoya, (J. Central med. Soc.)* No. 155. Abstr. in *Japan med. World*, 1921, **1,** 23 (W.L. 10880), and in *Trop. Dis. Bull.*, 1923, **20,** 237. (W.L. 21797)

VALLISNIERI, A., 1713.—Nuove osservazioni ed esperienze intorno all ovaja scoperta ne' vermi tondi dell' uomi, e de'vitelli con varie lettere spettanti alla Storia medica e naturale. Padova.

WAGNER, O., 1933.—"Immunisierungsversuche bie experimenteller Askaris-Infektion der Maus." *Z. ImmunForsch.*, **78,** 372–382. (W.L. 23456)

WALL, G. VAN DER, 1958.—"Zur Frage des pränatelen Spulwurmbefalls beim Schwein." *Tierärztl. Umsch.*, **13,** 48–50. (W.L. 21249a)

WETZEL, R., 1952.—"Helminthen und Immunität." *Zbl. Bakt.* (*Orig.*), **158,** 199–205. (W.L. 23684)

WHARTON, L. D., 1915.—"The development of the eggs of *Ascaris lumbricoides*." *Phillip. J. Sci. Sect. B. trop. Med.*, **10,** 19–23. (W.L. 16189)

WHITE, E. G., 1941.—"Chronic focal interstitial hepatitis in the pig." *Vet. J.*, **97,** 155–172. (W.L. 22518)

WIGGIN, N. J. B., 1955.—"Studies on conglutination with special reference to complement, anticomplementary action, and immunoconglutinin." *Ph.D. Thesis, University of Cambridge, England.*

YOSHIDA, S., 1919a.—"On the development of *Ascaris lumbricoides* L." *J. Parasit.*, **5,** 105–115. (W.L. 11428)

————, 1919b.—"On the migrating course of ascarid larvae in the body of the host." *Ibid.*, **6,** 19–27.

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 345–352.

On Two New Species of the Genus *Enterobius* Leach, 1853, from a Colobus Monkey

By S. Wahid, M.Sc.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The material described in this paper consists of ten mature males and ten females obtained from the intestine of a Colobus monkey (*Colobus* sp.) which died on its way to London, from West Africa. The specimens were collected at the London Zoo Prosectorium. On detailed study, they proved to belong to two different, hitherto unknown, species of the genus *Enterobius*. These two species differ markedly in the length and shape of the spicule and number of caudal papillae. There are six males and a number of females of one species, which is named *E. pesteri* n.sp and two males of another species, *E. inglisi* n.sp.

E. (*E.*) *pesteri* n.sp.

The males are very strongly coiled at their posterior extremities. The cuticle is striated transversely. The anterior end bears a small cuticular inflation which starts from the base of the lips. Later it forms narrow lateral alae throughout the body length. There are three fairly prominent lips which are neither incised at their bases nor reduced in size to give any asymmetrical appearance to the anterior extremity. The dorsal lip bears two large single papillae, each ventro-lateral lip has one single ventro-lateral papilla and a prominent laterally situated amphid. In addition, two nerves could be seen on each lip, near the papillae, running along towards the edge of the lips, but due to the condition of the specimens the presence of finer papillae could not be confirmed. The pharyngeal portions are very conspicuous and are separate anteriorly where they project into the buccal cavity. The portions appear square in the

end-on-view (Fig. 2). Interlabia are absent. The oesophagus is typically oxyurid, with a posterior bulb. The excretory pore opens at the level of the posterior bulb.

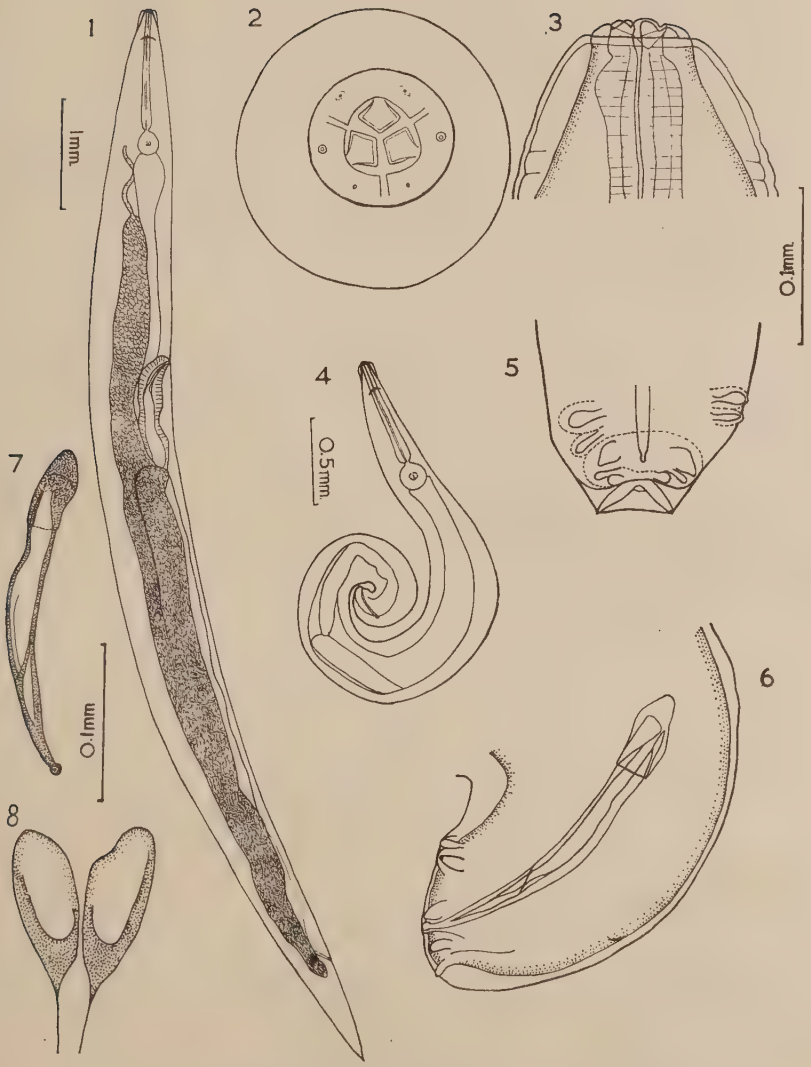
Male : The body measures 3·6–4·1 mm. in length and 0·34 mm. in maximum thickness. The width of the cephalic extremity at the level of the cuticular inflation is 0.075–0·094 mm. The cuticular inflation extends up to 0·13–0·165 mm. from the base of the lips. The oesophagus measures 0·72–0·82 mm. in length including the posterior bulb. The bulb measures 0·135–0·15 mm. and 0·15–0·165 mm. in width and length respectively. The nerve ring is situated at 0·17–0·2 mm. from the anterior extremity.

The tail is curved ventrally. There are six pairs of caudal papillae of which two large pedunculate pairs are preanal in position. The next three pairs are situated very close to each other. The last pair is also pedunculate, supporting the caudal alae. The spicule is rather thick and long, measuring 0·217–0·228 mm. It is 0·018 mm. wide at the middle of its length and ends in an oval knob-like structure posteriorly. It is highly characteristic in shape. The basal portion of the spicule is bifid when seen in ventral view (Fig. 8). There is a small rod-like cuticularised structure at the middle of the spicule, running diagonally (see Fig. 7).

Female : The length is 7·4–9·2 mm. and the maximum width is 0·5–0·63 mm. The width of the head at the level of the cuticular inflation is 0·097–0·105 mm. The cuticular inflation extends up to 0·165–0·18 mm. from the base of the lips. The oesophagus measures 0·99–1·3 mm. in length, including the posterior bulb which is 0·17–0·18 mm. and 0·18–0·21 mm. in width and length respectively. The nerve ring is situated at 0·24 mm. from the anterior extremity.

The vulva opens at 2·4–3·1 mm. from the anterior end and divides the body in a ratio of 1 : 2–2·1. The ovejector is muscular and runs posteriorly. The eggs are thin-shelled, slightly flattened on one side and measure 0·067–0·071 × 0·026–0·03 mm. in length and width respectively. The tail is relatively small and measures 0·7–0·75 mm. in length.

Relationships : In the structure of the anterior extremity this species differs from all the known species of this group. According to Inglis (1961) the pharyngeal portions are embedded in the mass of lips in the genus *Enterobius* and they project freely into the buccal cavity in the genus *Trypanoxyuris*. The present specimens are close to *Enterobius* in all the other characters ; the absence of a



Figs. 1–8. *Enterobius* (*E.*) *pesteri* n.sp.

1.—Female, lateral view. **2.**—En face view of the anterior extremity. **3.**—Anterior end. **4.**—Male, lateral view. **5.**—Male, posterior extremity, ventral view. **6.**—Male tail, lateral view. **7.**—Spicule in lateral view. **8.**—Basal part of the spicule, ventral view.

tail spike, the number of cephalic papillae, the shape of the spicule and the Old World distribution of the host, but differs in the character of the pharyngeal portions in which it resembles the genus *Trypanoxyuris* except that it has three large distinct lips, more characteristic of *Enterobius*. The significance of the shape of the basal portion of the spicule cannot be assessed because of insufficient knowledge of its detailed structure in the other species of *Enterobius*, but it appears to be more strongly developed than in *E.* (*E.*) *buckleyi* and *E.* (*E.*) *lerouxi* Sandosham, 1950, and less strongly developed than in *E. nycticebi* Baylis, 1928, and *E. lemuris* Baer, 1935, (see Inglis, 1961).

This is the first record of a species of Old World pin-worm in monkeys with distinctly free pharyngeal portions, but in all other characters it is a typical *Enterobius*. I propose the name *E.* (*E.*) *pesteri* n.sp. after Mr. F. R. N. Pester of the Department of Parasitology.

E. (*E.*) *pesteri* n.sp.

Host: Colobus monkey.

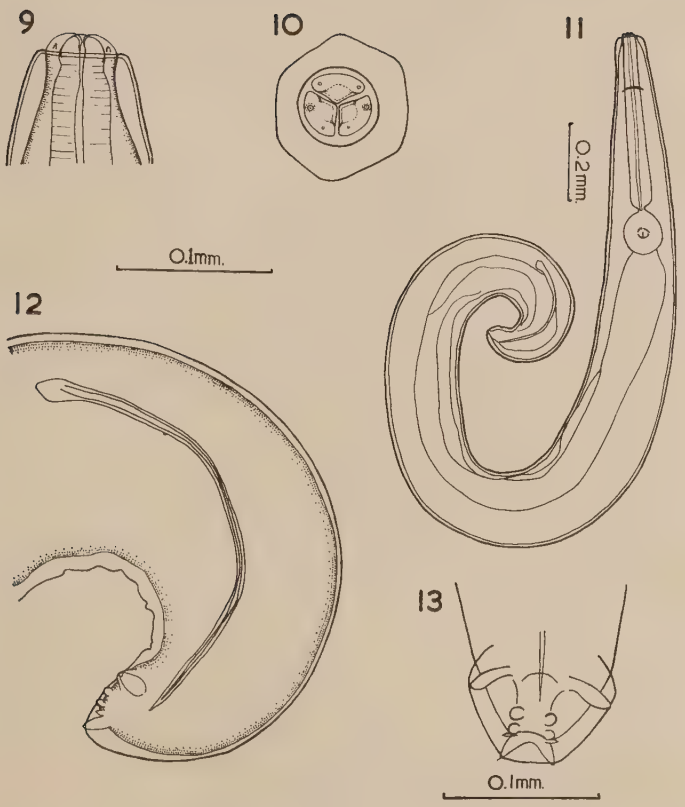
Location: Intestine.

Locality: W. Africa.

Enterobius (*E.*) *inglisi* n.sp.

Two males of this species were found together with the specimens of *E.* (*E.*) *pesteri*. Females were not present. Because of the difference in the anterior extremity of these two species it was easy to assign the female specimens to *E.* (*E.*) *pesteri* (Fig. 2). These are relatively smaller and thinner specimens. The posterior extremity is curved ventrally but not strongly coiled.

The cuticle is striated transversely. The anterior extremity bears three lips, one larger dorsal lip and two smaller ventro-laterals. The dorsal lip is provided with two single papillae, each ventro-lateral lip contains one single small papilla, ventro-lateral in position and one lateral amphid. The cephalic extremity bears a cuticular inflation for a short distance, which narrows to form lateral alae extending to the posterior end of the body. The oesophagus is relatively short, with a posterior bulb. The excretory pore opens at the level of the oesophageal bulb.



Figs. 9–13.—*E.* (*E.*) *inglis* n.sp.

9.—Anterior extremity. 10.—Anterior extremity, en face view. 11.—Male, lateral view. 12.—Male tail, lateral view. 13.—Posterior extremity of male, ventral view.

Male : The body length is 3–3·2 mm. and the maximum width is 0.2 mm. The cuticular inflation extends up to 0·075 mm. in length from the base of the lips. The width of the cephalic extremity at the level of the cuticular inflation is 0·086 mm. The pharyngeal portions are embedded in the mass of the lips. The oesophagus measures 0·57 mm. in length including the posterior bulb which measures 0·105 mm. in length and 0·09 mm. in width. The nerve ring is located at 0·135 mm. from the anterior extremity.

The spicule is very long and narrow with a pointed tip. It measures 0·3 mm. in length including a small cuticularised basal portion which measures about 0·03 mm. in length. The width of the spicule at the middle of its length is 0·005 mm. Because of the lack of specimens, the shape of the basal part of the spicule could not be studied in dorso-ventral view, its lateral view is shown in Fig. 12. There are five pairs of caudal papillae of which the first and the last pairs are large and pedunculate, supporting the caudal cuticular expansion. The second and third pairs are medium sized, round and sessile ; the fourth pair is very small and is situated between the second sessile pair and the last pedunculate pair.

Relationships : The present specimens differ from all the known species of the genus *Enterobius* in the length of the spicule which is very long. In *E. zakiri* Siddiqi and Mirza, 1954, the spicule is 0·35 mm. long which is the longest for this genus, but the description given by the above authors is very unsatisfactory, so that a full comparison is not possible. *E.* (*E.*) *inglisi* can also be easily distinguished from other known species by the shape of the spicule and the arrangement of the caudal papillae. Cameron's (1929) description of *E. pitheci* is of females only, but this species is from a different host and also has a different geographical distribution. If, as suggested by Cameron, the specimens referred to *E. bipapillatus* by Baylis (1923) really belong to *E. pitheci*, then it can be distinguished from the present specimens by the size of the spicule which is only 60μ in the specimens of Baylis.

Hence the present worms are considered to be new, and are named *E. inglisi* n.sp. after Dr. W. G. Inglis (of the Nematode Section) of the British Museum, Natural History.

Host : Colobus monkey.

Location : Intestine.

Locality : W. Africa.

The two species of *Enterobius* described above were recovered from a Colobus monkey which was in captivity for only a short time. It was brought to England from West Africa by sea, with other monkeys, and the voyage took nearly a month. It is difficult to say definitely whether the occurrence of two species in the same monkey is due to some secondary infection during its journey or whether it is due to their both occurring naturally in the wild hosts.

The two species *E. lagothricis* and *E. duplicidens* Buckley, 1931, were also described from a single animal, a Woolly Monkey, *Lagothrix humboldtii*, although Buckley pointed out that the structural differences were probably enough to warrant the erection of a new genus. Since then, these two species were transferred to *Buckleyenterobius* Sandosham, 1950, and later to *Trypanoxyuris* Vevers, 1923, by Inglis and Diaz-Ungria, 1959. Other exceptions to the rule that a single species of *Enterobius* is confined to a single genus of Primate host are the records of *E. vermicularis* (Linnaeus, 1785) from the Chimpanzee, the Lar Gibbon and the Lion Marmoset (Sandosham, 1950).

Acknowledgments

I wish to express my gratitude to Professor J. J. C. Buckley, under whose guidance this work was carried out, and to Dr. W. G. Inglis for his kind help. Also, I am very grateful to the staff of the Prosectorium of the London Zoo from whom the material was received.

References

Baer, J. G., 1935.—"Etude de quelques helminthes de Lémuriens." *Rev. suisse Zool.*, **42**, 275–291. (W.L. 19288)

Buckley, J. J. C., 1931.—"On two new species of *Enterobius* from the monkey *Lagothrix humboldtii*." *J. Helminth.*, **9**, 133–140. (W.L. 11224c)

Cameron, T. W. M., 1929.—"The species of *Enterobius* Leach in Primates." *ibid.*, **7**, 161–182.

Inglis, W. G., 1961.—"The Oxyurid parasites (Nematoda) of Primates." *Proc. Zool. Soc. Lond.*, **136**, 103–122. (W.L. 16969b)

———, and Diaz-Ungria, C., 1959.—"Nematodes de Venezuela, III. Nematodes parasitos vertebrados Venezolanos. I. Una revision del genero *Trypanoxyuris* (Ascaridina Oxyuridae)." *Mem. Soc. Ciencias Nat. La Salle*, **19**, 176–212. (W.L. 13480a)

Sandosham, A. A., 1950.—"On *Enterobius vermicularis* (Linnaeus, 1785) and some related species from Primates and Rodents." *J. Helminth.*, **24,** 171–204. (W.L. 11224c)

Siddiqi, A. H., and Mirza, M. B., 1954.—"On a new oxyurid worm, *Enterobius zakiri* n.sp. from the rectum of *Semnopithecus schistaceus* (Tarai langur)." *Indian J. Helminth.*, **6,** 24–26: (W.L. 9939g)

Solomon, S. G., 1933.—"On a new species of *Enterobius* from the Marmoset (*Callithrix jacchus*)." *J. Helminth., II* **1,** 95–100. (W.L. 11224c)

Journal of Helminthology, Vol. XXXV, Nos. 3/4, 1961, *pp.* 353–360.

On *Amplicaecum iguanae* n.sp. with Notes on *Hexametra sewelli, Kalicephalus micrurus* and *Viguiera euryoptera**

By S. Wahid, M.Sc.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

One male and three female specimens belonging to the genus *Amplicaecum* were present in a collection of nematodes in the British Museum, Natural History, made available to me through the courtesy of Dr. W. G. Inglis.

Amplicaecum Baylis, 1920

Amplicaecum iguanae n.sp.

These are medium-sized slender worms. The females are slightly larger than the males. The cephalic end bears three prominent square-shaped lips, one dorsal and two latero-ventral in position. The inner surface of the margin of the lips is dentate ; the denticles are fine but prominent. The dorsal lip possesses two double papillae and each ventro-lateral lip is provided with one simple and one double papilla. Relatively small interlabia are present. The interlabial grooves are well marked and surround the base of each lip on its outer surface, forming a collar-like structure. The oesophagus is muscular and has no oesophageal appendix. A narrow intestinal caecum is present, which is less than $\frac{1}{3}$rd of the length of the oesophagus. The cuticle is finely striated transversely.

Male. The male is 19 mm. long and 0·45 mm. in maximum width. The diameter of the head is 0·15 mm. The oesophagus measures 1·7 mm. in length. The intestinal caecum is 0·51 mm. long. The nerve ring encircles the oesophagus at a distance of 0·45 mm. from the head end.

The tail is curved ventrally and the cuticle in the caudal region is slightly expanded though no clear caudal alae are present. The spicules are equal and very small (0·26 mm.) ; they are rod-like with blunt tips. There are about 30 pairs of preanal papillae arranged laterally in two longitudinal rows and a very inconspicuous median

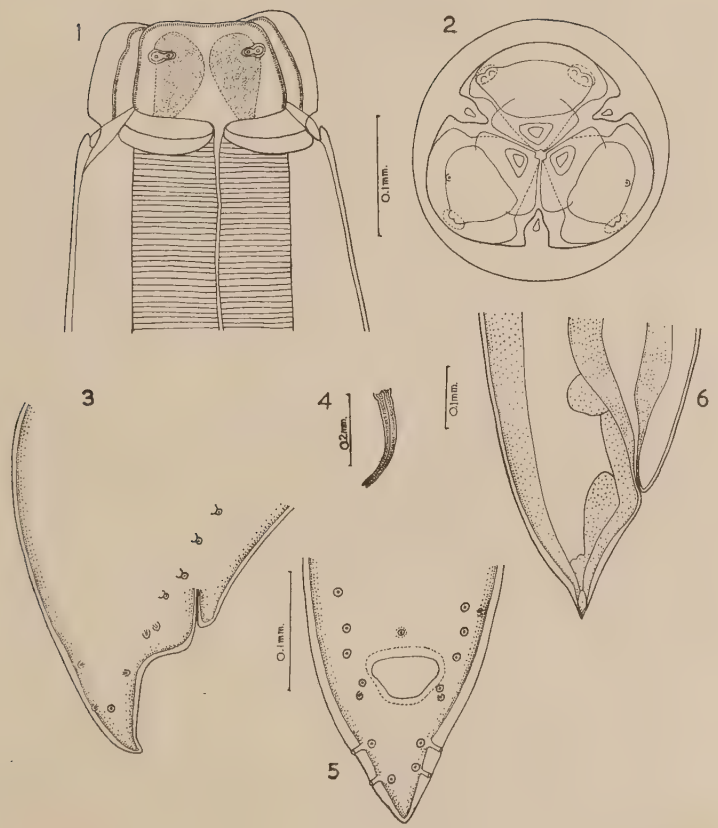
* Part of a thesis approved by the University of London for the award of the M.Sc. degree.

TABLE I

Comparison of *A. iguanae* n.sp. with closely related species

	A. varani Baylis and Daubney, 1922		*A. cacopi* Chatterji, 1936		*A. schoutedeni* Baylis, 1940		*A. monitor* Khera, 1954		*A. ranae* Gupta, 1959		*A. iguanae* n.sp.	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Body length	22·2—24·9	24·75	11·4—18·6	14—34·5	18–35	37·5—45	32–36	40–50	18·8—20·96	Unknown	19	20–23
Length of the oesophagus	3·5—4·7		1·9—2·5	1·96—3·5	2·3—2·4	2·6—3·47	3·5—3·7	3·9—4·1	2·36		1·77	2·4
Length of the intestinal caecum	0·9—1		1·0—1·5	1·14—2·1	1·3—1·4	1·65—2·3	0·7	0·9	1·16		0·51	0·72
Length of spicules	0·5		0·21—0·28		0·55—0·6		0·32–0·4 & 0·36—0·5 (subequal)		0·2–0·22 & 0·19—0·2 (subequal)		0·263	
Male caudal papillae	5 pairs postanal 1 median 32 pairs preanal		4 pairs postanal 5 pairs preanal		4 pairs postanal 1 median 13–15 prs. preanal		5 pairs postanal 23–28 prs. preanal		4 pairs postanal 5 pairs preanal		6 pairs postanal 1 median 30 pairs preanal	
Position of vulva from anterior end	6·5		5–11·4		13–14		10·4		—		5·2	
Host	*Varanus salvator*		*Cacopus systoma*		*Varanus niloticus*		*Varanus monitor*		*Rana tigrina R. cancrivora*		*Iguana* sp.	

All measurements in mm.



Amplicaecum iguanae n.sp.

Fig. 1.—Anterior extremity, dorsal view. Fig. 2.—*En face* view of anterior end. Fig. 3.—Male posterior extremity, lateral view. Fig. 4.—Spicules. Fig. 5.—Male posterior extremity, ventral view. Fig. 6.—Female tail, lateral view.

papilla just anterior to the cloaca. The postanal papillae comprise six pairs arranged as follows : two pairs of papillae just posterior to the cloaca, two pairs almost equidistant from the second pair of

postanal papillae which are sublateral in position and two pairs of papillae at about the same level as the 3rd and 4th pair, but lateral in position.

Female. The females measure 20–23 mm. in length and 0·51 mm. in maximum width. The diameter of the head is 0·17–0·18 mm. The oesophagus is 2·4 mm. long in a female of 21 mm. length. The intestinal caecum is thin and 0·72 mm. long. The nerve ring is situated at 0·61 mm. from the anterior end. The vulva opens anterior to the middle of the body at a distance of 5·2 mm. from the anterior end. The vagina is muscular and runs towards the posterior extremity to join the common uterus which is formed by two uteri.

Amplicaecum iguanae n.sp. is close to *A. varani* Baylis and Daubney, 1922, and *A. monitor* Khera, 1954, in the number of preanal papillae. It differs from *A. varani* Baylis and Daubney, 1922, in the smaller length of the oesophagus, the length of the intestinal caecum, in the length of the spicules which are almost half as long as those of *A. varani*, and in the number and arrangement of the male caudal papillae. In *A. varani* there are five pairs of postanal papillae while in *A. iguanae* there are six pairs. It differs from *A. monitor* Khera, 1954, in the presence of an unpaired median papilla on the anterior lip of the cloaca which is absent in Khera's species, in having equal spicules (which are subequal in *A. monitor*) and in the number and arrangement of the male postanal papillae. *A. iguanae* also differs from *A. monitor* in having a considerably smaller body length.

Gupta, S. P. (1959) described *A. ranae* from East Pakistan which was collected from *Rana tigrina* and *R. cancrivora*. This species is devoid of interlabia and the spicules are subequal. the number of postanal papillae in *A. ranae* is four pairs and the preanals five pairs with one median unpaired papilla whereas there are six pairs of postanal papillae and thirty pairs of preanals in the present worm.

In Table I, *A. iguanae* is compared with closely related species of the genus *Amplicaecum*. As it differs from all the known species of the genus *Amplicaecum* it is regarded as new and is named *A. iguanae* n.sp.

Host : *Iguana* sp.

Location : Intestine

Locality : India.

Kalicephalus Molin, 1861

Kalicephalus micrurus (Daubney, 1923) Yorke and Maplestone, 1926

Synonym : *Diaphanocephalus micrurus* Daubney, 1923

The body is attenuated towards the posterior extremity and the cuticle is finely striated transversely. The mouth opening is elongated and not markedly tilted towards the dorsal side.

Male. The male is 8–9·4 mm. long and 0·28–0·3 mm. in width. The buccal capsule is 0·135 mm. long and 0·15 mm. wide at its base. The distance between the anterior extremity and the nerve ring is 0·18 mm. The excretory pore opens at the level of the posterior end of the oesophagus, at 0·35 mm. from the cephalic end. The oesophagus is 0·3 mm. long, the diameter of the bulb is 0·15 mm.

The spicules are equal, alate and measure 0·435–0·45 mm. in length and the alae form a spoon-shaped dilatation at the tip. A gubernaculum is present which measures 0·17–0·18 mm. in length and the telamon measures 0·09 mm. The bursa is of the normal type as described for this genus (Figs. 9 and 12).

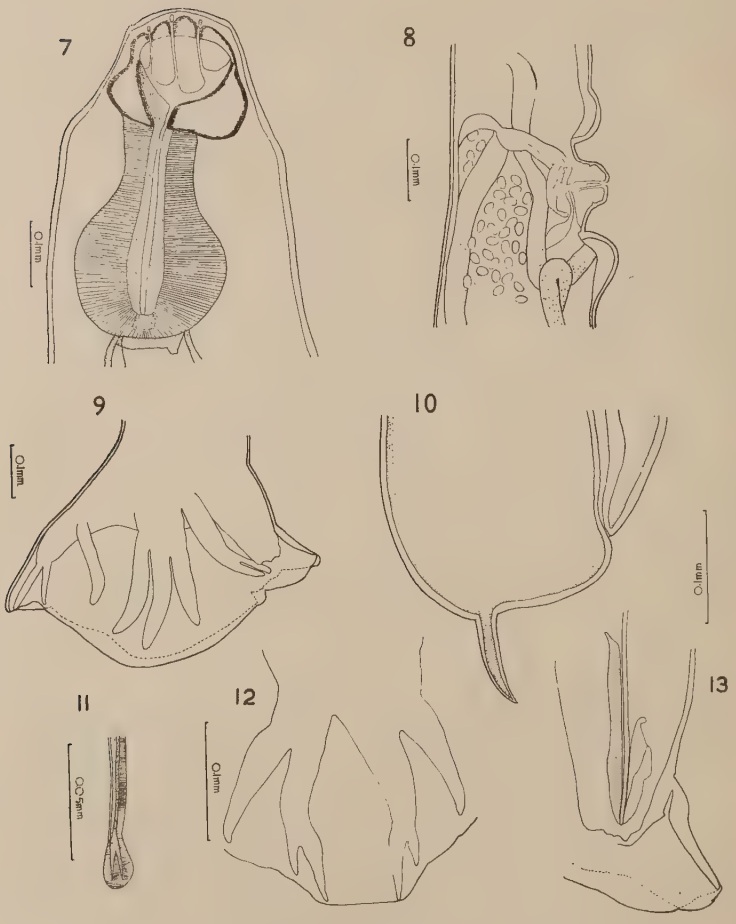
Female. The female is 11–13·5 mm. long and 0·48 mm. wide and its buccal capsule is 0·166 mm. long at the centre and 0·225 mm. wide at the base. The nerve ring is at 0·24 mm. and the excretory pore at 0·48 mm. from the cephalic end. The length of the oesophagus is 0·33 mm. and the diameter of the posterior bulb is 0·2 mm. The position of the vulva is variable. It is situated posterior to the middle of the body, at a distance of 1·83–3·5 mm. from the posterior extremity. The uteri are convergent and the eggs measure 0·06–0·04 mm. The anus is subterminal. The tail is rounded and ends in a sharply pointed process at its tip.

K. micrurus was originally described from the intestine of *Macrelaps microlepidotus* by Daubney (1923) as *Diaphanocephalus micrurus*. The present specimens from a Herald snake closely resemble *K. micrurus* except for a slight difference in the length of the spicules. The spicules were described by Daubney as 0·36–0·38 mm. long whereas they are 0·435–0·45 mm. in the present specimens. The present material is recorded from a new host.

Host : *Crotaphopeltis hotamboeia* (Herald snake)

Location : Intestine

Locality : Nyasaland.



Kalicephalus microrus (Daubney, 1923) Yorke and Maplestone, 1926

Fig. 7.—Anterior extremity, lateral view. Fig. 8.—Vulva. Fig. 9.—Bursa, lateral view. Fig. 10.—Female tail, lateral view. Fig. 11.—Posterior part of the spicule. Fig. 12.—Branches of dorsal ray of bursa. Fig. 13.—Gubernaculum and telamon.

Viguiera Seurat, 1913

Viguiera euryoptera (Rudolphi, 1819) Seurat, 1913

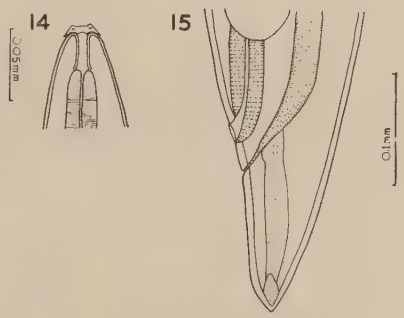
Two males and two females of this species were collected from *Dendrocitta vagabanda* in Lahore (Pakistan). This species was described for the first time from India by Singh, 1949 and also from a new host *Dicrurus coerulescens*. The present specimens agree with the previous descriptions in almost all respects. Here it is recorded from a new host *Dendrocitta vagabanda* (Indian tree pie) from Pakistan.

One more species *V. leiperi* was added to this genus by Ali, 1954, from *Tchitrea paradisi* (Paradise fly-catcher) in Hyderabad, India.

Host: *Dendrocitta vagabanda*

Location : Gizzard

Locality : Pakistan.



Viguiera euryoptera (Rudolphi, 1819) Seurat, 1913

Fig. 14.—Anterior extremity, lateral view, female. Fig. 15.—Posterior extremity lateral view, female.

Hexametra Travassos, 1919

Hexametra sewelli (Baylis and Daubney, 1922) Mosgovoy, 1951

Synonym : *Polydelphis sewelli* Baylis and Daubney, 1922

One male and one female specimen of this species were collected from *Psammophis subtaeniatus*. These specimens are very close to *H. sewelli* in all the characters except in the number of postanal

papillae. In the original description the number is 5 pairs of papillae and a pair of phasmids and in the present specimen there are only 4 pairs of papillae and a pair of phasmids. It differs from all the other known species of the genus *Hexametra*. As the arrangement of the papillae and other characters are very similar to *H. sewelli* and only one male specimen is present, here it is regarded as the same worm.

H. sewelli was well described originally from the Montpellier snake (*Coelopeltis monspessulana*) from India, by Baylis and Daubney, 1922, as *Polydelphis sewelli*. It is also recorded from *Tropidonotus stolatus*, *T. piscator* and *Bungarus fasciatus* from India. Here it is recorded from *Psammophis subtaeniatus* from Rhodesia for the first time and also from a new host.

Host : *Psammophis subtaeniatus*

Location : Intestine

Locality : Rhodesia.

References

Ali, S. M., 1954.—"On a new species of the nematode genus *Viguiera* from the paradise fly-catcher." *Proc. Indian Sci. Congr.*, **41,** 32. (W.L. 16755)

Baylis, H. A., 1920.—"On the classification of the Ascaridae. 1. The systematic value of certain characters of the alimentary canal." *Parasitology*, **12,** 253–264. (W.L. 16035)

———, 1921.—"On the classification of the Ascaridae. II. The Polydelphis group with some account of other Ascarids parasitic in snakes." *Ibid.*, **12,** 411–426. (W.L. 16035)

———, 1936.—Nematoda, Vol. I (Ascaroidea and Strongyloidea). The Fauna of British India. London. 408 pp. (Taylor and Francis).

———, 1940.—"On a further collection of parasitic worms from the Belgian Congo." *Ann. Mag. nat. Hist.*, ser. 11, **29,** 401–417. (W.L. 1050)

———, and Daubney, R., 1922.—"Report on the Parasitic Nematodes in the Collection of the Zoological Survey of India." *Mem. Indian Mus.*, **7,** 263–347. (W.L. 13401)

Chatterji, R. C., 1936.—"On a new species of nematode *Amplicaecum cacopi* n.sp. from *Cacopus systoma*." *Ann. trop. Med. Parasit.*, **30,** 41–44. (W.L. 1063)

Daubney, R. 1923.—"Note on the genus *Diaphanocephalus* (Nematoda : Strongylidae) parasitic in Reptiles, with a description of three new species." *Parasitology*, **15,** 67–74. (W.L. 16035)

Gupta, S. P., 1959.—"Nematode parasites of vertebrates of East Pakistan. II. *Amplicaecum ranae* n.sp. (Heterocheiliidae Railliet & Henry, 1915) from Amphibia." *Canad. J. Zool.*, **37,** 477–479. (W.L. 5898c)

Khera, S. 1954.—"Nematode parasites of some Indian vertebrates." *Indian J. Helminth.*, **6,** 27–113. (W.L. 9939g)

Singh, S. N., 1949.—"Studies on the helminth parasites of Birds in Hyderabad State. Nematoda III." *J. Helminth.*, **23,** 25–58. (W.L. 11224c)

Yorke, W. and Maplestone, P. A., 1926. The Nematode Parasites of Vertebrates. London. 536 pp.

Subject Index to Volume XXXV

PAGE

Agama cyanogaster host of *Megacapsula* n.sp. 169
Amplicaecum iguanae n.sp. from iguana 353
Arostellina reticulata from *Clytolaema* sp. redescribed... ... 174
Ascaris suum, antibody response to infection with, in pigs ... 333
transfer of immunity of, in guineapigs ... 315
vitulorum, bacteriostatic action of 226
Aviabronema new subgenus, key to species of 31
parasites of birds 11
Bacteriostatic properties of helminths 225
Bertiella studeri infecting man in Malaya 207
Boar, wild, host of *Oesophagostomum watanabei* n.sp.... ... 203
Brugia buckleyi n.sp. from *Lepus* sp. in Ceylon 209
patei, development and behaviour in mosquitoes ... 285
Brugiella new subgenus for *Brugia buckleyi* n.sp. 213
Bulinus cernicus, host of *Paramphistomum* n.sp. 80
forskalii, host of *Paramphistomum* n.sp. 80
senegalensis, host of *Paramphistomum* n.sp. 80
Butastur, host of *Habronema* n.sp. 28
Buzzard eagle, host of *Physaloptera* n.sp. 50
Capillaria incrassata, from *Neomys fodiens*, redescribed ... 66
oesophagicola from *Neomys fodiens*, redescribed ... 61
Catfish, marine, host of *Elongoparorchis* n.sp. 30
Cercaria cristocorpa n.sp. from *Valvata piscinalis* 139
edgwarensis n.sp. from *Planorbis* sp. in Britain ... 278
kenilworthensis n.sp. from *Planorbis* sp. in Britain ... 275
kentensis n.sp., from *Lymnaea pereger* 134
Chaetogaster limnaei as predator on miracidia 269
Choanotaenia hypoleucia transferred to *Kowalewskiella* ... 178
Cotylophoron cotylophorum, development of 161
pathogenicity of 165
Coucal, host of *Cyrnea* n.sp. 41
Ctenocephalides felis, host of *Dipetalonema* n.sp. 149

PAGE

Cyrnea singhi n.sp. from *Centropus* sp. 41
Daubaylia elegans n.sp. from freshwater snail 115
Delopsylla crassipes host (?) of *Dipetalonema* n.sp. 157
Development of *Paramphistomum* n.sp. in snail host... ... 82
Dipetalonema manson-bahri n.sp., development in fleas ... 149
from *Pedetes* sp. 143
microfilaria of 146
Eagle, tawny, host of *Habronema monoptera* 21
Echinostoma londonensis n.sp., adult of *Cercaria londonensis* Khan, 1960 119
from pigeon (experimental) ... 119
life cycle of 119
Elongoparorchis pneumatis gen. et sp. nov., from *Arius jella*... 301
Enterobius inglisi n.sp. from *Colobus* sp. 348
pesteri n.sp. from *Colobus* sp. 345
Excisa buckleyi n.sp. from *Dissoura* sp.... 38
Falcon, Indian, host of *Habronema* n.sp. 20
Fasciola gigantica, bacteriostatic action of 226
Fish, freshwater, host of *Orientocreadium* n.sp. 259
Raphidascaroides n.sp. 262
Fleas, hosts of *Dipetalonema* n.sp. 149
Grackle, host of *Habronema* n.sp. 24
Habronema ameerae n.sp. from *Cerchneis* sp. 14
chabaudi n.sp., from *Falco* sp. 20
daleri n.sp., from *Crocopus* sp. 22
hyderabadensis n.sp. from *Gracula* sp. 24
key to species of 30
magnipapillatum n.sp. from *Butastur* sp. 28
mansioni, new host record in *Circus macrourus* ... 12
redescribed 12
monoptera redescribed from *Aquila* sp. 21
new subgenus, parasites of mammals 11
singhi n.sp., from *Milvus* sp. 17
suraiyae n.sp., from *Falco* sp. 15
waheedae n.sp., from *Haliastur* sp.... 26
Hare, Ceylon, host of *Brugia buckleyi* n.sp. 209

PAGE

Harrier, host of *Physaloptera* sp. 50
Indian, new host of *Habronema mansioni* 12
Hexametra sewelli from new host, *Psammophis* sp. 360
locality, Rhodesia 360
Hoopoe, host of *Sicarius dipterum* 44
Humming bird, new host for *Arostellina reticulata* 177
Hydatid cyst, in goat in Ceylon 221
unusual brood capsule in 221
Ibis, Sacred, host of *Paradilepis* n.sp. 255
Iguana, host of *Amplicaecum* n.sp. 353
Indoplanorbis exustus, development of *Cotylophoron* in ... 161
Kalicephalus micrurus from *Crotaphopeltis* sp., redescribed ... 357
Kestrel, Indian, host of *Habronema* n.sp. 14
Key to species of *Aviabronema* n. subgen. 31
Habronema n. subgen. 30
Labiduris 97
Physaloptera in birds... 59
subgenera of *Habronema* 11
Kite, brahmany, host of *Habronema* n.sp. 26
Indian, host of *Habronema* n.sp. 17
Physaloptera sp. 49
Labiduris africana from *Kinixys* spp. redescribed 91
Life cycle of *Echinostoma londonensis* n.sp. 119
Lymnaea pereger host of *Cercaria kentensis* n.sp. 134
Mansonia uniformis as host for *Brugia patei* 285
Megacapsula leiperi gen. et sp. nov. from *Agama* sp. ... 169
Merlin, red headed, host of *Habronema* n.sp. 15
Miracidia destroyed by *Chaetogaster limnaei* 269
Monkey, *Colobus*, host of *Enterobius* spp. 345
Nematodes, recovery of, by "Mistifier" 389
Nippostrongylus brasiliensis, behaviour in different animals ... 109
Oesophagostomum watanabei n.sp. from *Sus leucomystax* ... 203
Orientocreadium lazeri n.sp. from *Clarias* sp. in Sudan ... 259
Oochoristica zonuri from *Psammophis* sp., new host 172
Pallas' fishing eagle, host of *Physaloptera* n.sp. 55
Paradilepis maleki n.sp. from *Treskiornis* sp. in Sudan ... 255

PAGE

Paramphistomum phillerouxi n.sp., from African cattle ... 76
relationships of 76
Physaloptera alata from *Milvus* sp. 49
alata chevreuxi from *Circus* sp. 50
indiana n.sp. from *Butastur* sp. 50
key to species in birds 59
microcauda n.sp., from *Pseudogyps* sp. 54
sultanae n.sp. from *Haliaetus* sp.... 55
Pie, Indian tree, new host for *Viguiera euryoptera* 359
Pigeon, green, host of *Habronema* n.sp. 22
Planorbis carinatus, host of *Daubaylia* n.sp. 115
corneus, host of *Echinostoma londonensis* n.sp. ... 119
planorbis, host of *Cercaria* n.spp. 275
Polystoma integerrimum, discussion and summary 181
Pouched rat, Gambian, host of *Skrjabinotaenia* n.sp. ... 233
Psammophis sibilans new host for *Oochoristica zonuri* ... 172
Pulmoverminae n. subfam. for *Pulmovermis* and *Hydrophitrema* 305
Pulmovermis and *Hydrophitrema* differentiated 306
Raphidascaroides bishaii n.sp. from *Gymnarchus* sp. in Sudan 262
Shrew, British water, host of *Capillaria incrassata* 66
oesophagicola ... 61
Sicarius dipterum from *Upupa* sp. redescribed 44
Skrjabinotaenia cricetomydis n.sp. from *Cricetomys* sp. in Nigeria 233
spp. regrouped 242
Snake, Herald, new host for *Kalicephalus micrurus* 357
Peter's Long-line, new host for *Hexametra sewelli* ... 360
Spring-hare, host of *Dipetalonema* n.sp. 143
Stork, white necked, host of *Excisa* n.sp. 38
Technique for recovery of nematodes 309
Tortoises, hosts of *Labiduris africana* in Nyasaland 91
Valvata piscinalis, host of *Cercaria cristocorpa* n.sp. 139
Viguiera euryoptera from new host, *Dendrocitta* sp. 259
Vulture, Indian, host of *Physaloptera* n.sp. 54
Xenopsylla cheopis host of *Dipetalonema* n.sp. 149

Index of Authors, Volume XXXV

PAGE

Ali, S. M. 1, 49

Bernard, J. 61

Desowitz, R. S., Wong, H. J. and Fernando, M. A. ... 207

Dinnik, J. A. 69

Dissanaike, A. S. and Paramananthan, D. C. ... 209, 221

Fitzsimmons, W. M. 91

Gharib, H. M. 101, 109, 225

Hockley, A. R. 233

Honer, M. R. and Jansen, J. 115

Khalil, L. F. 255, 259, 263, 269

Khan, D. 119, 133, 275

Laurence, B. R. and Pester, F. R. N. 285

Nelson, G. S. 143

Rao, K. H. 301

Sandars, D. F. 305

Sturrock, R. F. 309

Taffs, L. F. 315, 319

Varma, A. K. 161

Wahid, S. 169, 345, 353

Williams, J. B. 181

Yamaguti, S. 203

New Names in Volume XXXV

New Subfamily

PAGE

PULMOVERMINAE Sandars, 1961 305

New Genera

ELONGOPARORCHIS Rao, 1961 301
MEGACAPSULA Wahid, 1961 169

New Subgenera

AVIABRONEMA Ali, 1961 11
BRUGIELLA Dissanaike and Paramananthan, 1961 ... 209
HABRONEMA Ali, 1961 11

New Species

AMPLICAECUM IGUANAE Wahid, 1961 353
BRUGIA BUCKLEYI Dissanaike and Paramananthan, 1961 209
CERCARIA CRISTOCORPA Khan, 1961 139
CERCARIA EDGWARENSIS Khan, 1961 278
CERCARIA KENILWORTHENSIS Khan, 1961 275
CERCARIA KENTENSIS Khan, 1961 134
CYRNEA SINGHI Ali, 1961 41
DAUBAYLIA ELEGANS Honer and Jansen, 1961 ... 115
DIPETALONEMA MANSON-BAHRI Nelson, 1961 ... 143
ECHINOSTOMA LONDONENSIS Khan, 1961 119
ELONGOPARORCHIS PNEUMATIS Rao, 1961 ... 301
ENTEROBIUS INGLISI Wahid, 1961 348
ENTEROBIUS PESTERI Wahid, 1961 345
EXCISA BUCKLEYI Ali, 1961 38
HABRONEMA AMEERAE Ali, 1961 14

PAGE

HABRONEMA CHABAUDI Ali, 1961 20
HABRONEMA DALERI Ali, 1961 22
HABRONEMA HYDERABADENSIS Ali, 1961... ... 24
HABRONEMA MAGNIPAPILLATUM Ali, 1961 ... 28
HABRONEMA SINGHI Ali, 1961 17
HABRONEMA SURAIYAE Ali, 1961 15
HABRONEMA WAHEEDI Ali, 1961 26
MEGACAPSULA LEIPERI Wahid, 1961 169
OESOPHAGOSTOMUM WATANABEI Yamaguti, 1961 203
ORIENTOCREADIUM LAZERI Khalil, 1961 259
PARADILEPIS MALEKI Khalil, 1961 255
PARAMPHISTOMUM PHILLEROUXI Dinnik, 1961 ... 70
PHYSALOPTERA INDIANA Ali, 1961 50
PHYSALOPTERA MICROCAUDA Ali, 1961 54
PHYSALOPTERA SULTANAE Ali, 1961 55
RHAPHIDASCAROIDES BISHAII Khalil, 1961 ... 263
SKRJABINOTAENIA CRICETOMYDIS Hockley, 1961 233

Corrigenda in Vol. XXXIV

Page 100—Line 20—delete "therefore".

Page 207—Line 9—for "vaseline" read "Vaseline Brand Petroleum Jelly".

Actual Dates of Publication for Volume XXXIV

Numbers 1/2 ... ... 13th October 1960

Numbers 3/4 ... ... 31st December 1960

JOURNAL OF HELMINTHOLOGY

SUPPLEMENT

IN HONOUR OF

R. T. Leiper

ON THE OCCASION OF HIS

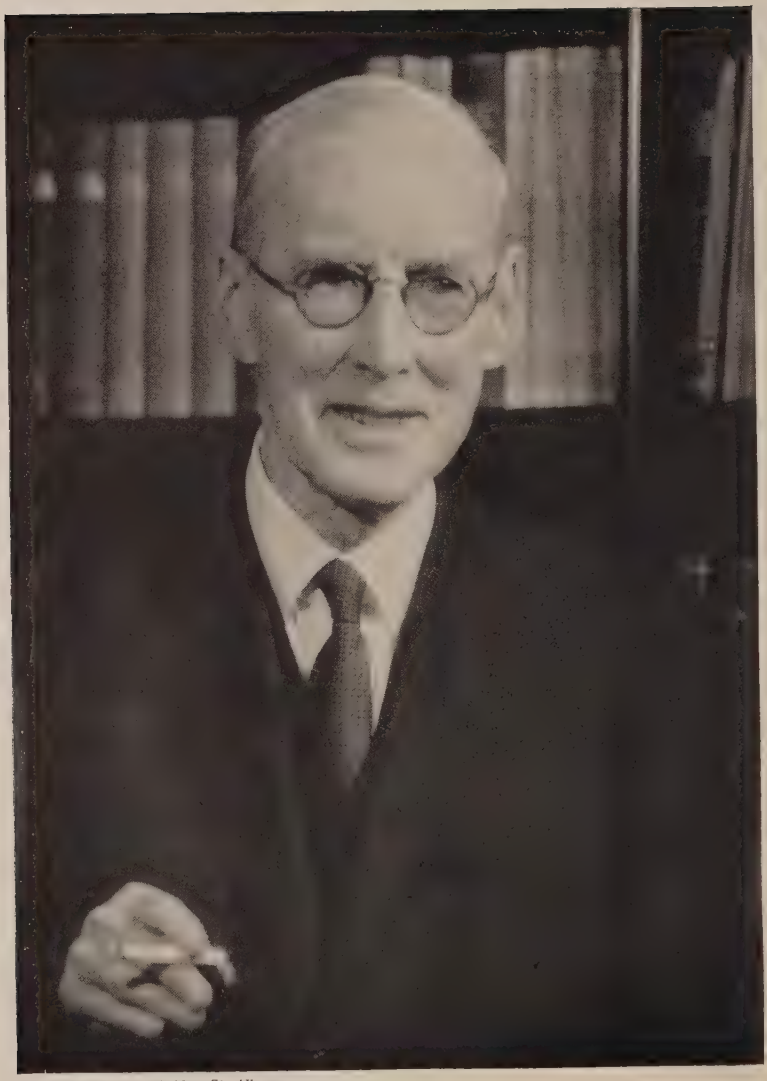
80th Birthday

1961

Acknowledgments and sincere thanks are tendered to all those who have contributed articles to this "festschrift" and to others who have helped in its construction. Special thanks are due to Mrs. Joy Gordon for her charmingly topical illustrations (pages v to xi) and to the printers for their close co-operation and excellent workmanship.

Editor.

Printed by Richard Bates Limited
Manchester, England



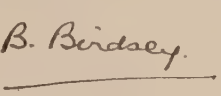
Photograph by Juliet Haddon, St. Albans

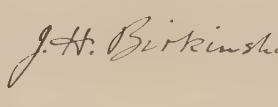
Yours
Octogenarianly
R. T. Leiber

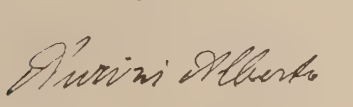
Greetings

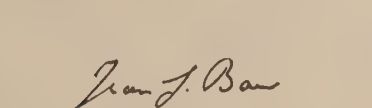
to R. T. Leiper

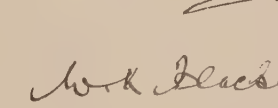
ON HIS EIGHTIETH BIRTHDAY

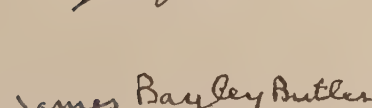


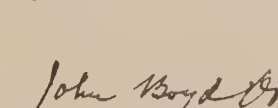


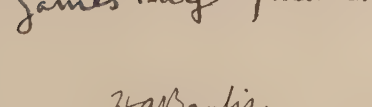


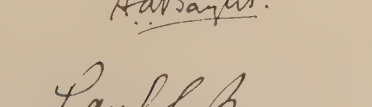


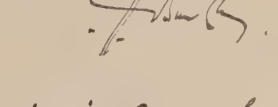


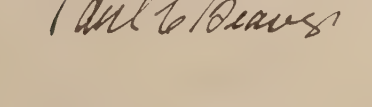


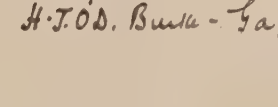


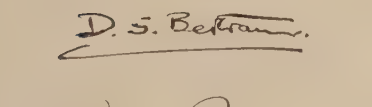


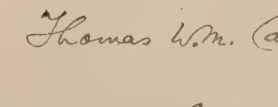






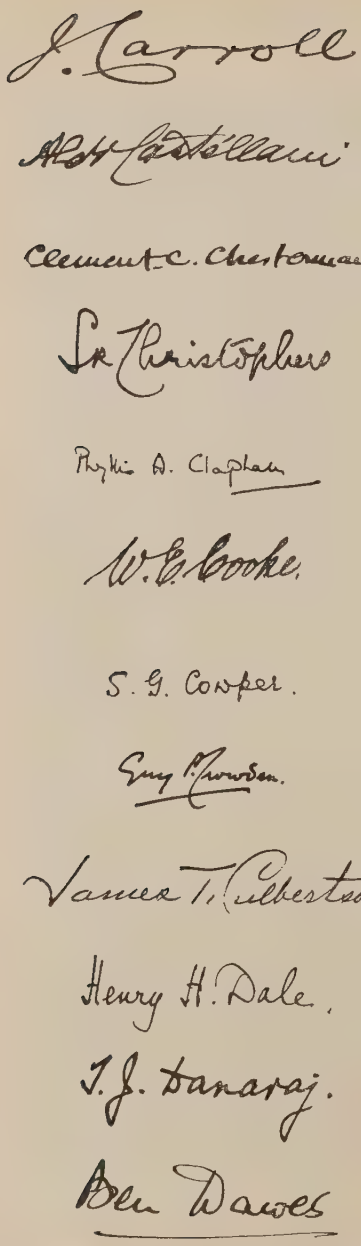






First Column : Syed Mehdi Ali ; Stanley Alstead ; Alberto Aurizi ; Jean G. Baer ; James Bayley Butler ; H. A. Baylis ; Paul C. Beaver ; D. S. Bertram ; Ettore Biocca.

Second Column : B. Birdsey ; J. H. Birkinshaw ; B. Bisseru ; W. K. Blackie ; John Boyd Orr ; J. J. C. Buckley ; H. J. O'D. Burke-Gaffney ; Thomas W. M. Cameron ; D. F. Cappell.



First Column : J. Carroll ; Aldo Castellani ; Clement C. Chesterman ; S. R. Christophers ; Phyllis A. Clapham ; W. E. Cooke ; S. G. Cowper ; Guy P. Crowden ; James T. Culbertson ; Henry H. Dale ; T. J. Danaraj ; Ben Dawes.

Second Column : M. E. Delafield ; L. M. Dennis ; J. A. Dinnik ; N. N. Dinnik ; P. Granville Edge ; E. E. Edwards ; N. Hamilton Fairley ; A. Murray Fallis ; Ernest Carroll Faust ; D. Fenwick ; A. C. Fisher ; A. E. Fountain.

Mary T. Franklin

Henri Galliard

P. C. C. Garnham

Joyce Gear.

V. J. Glanville

J. B. Goodey

Hugh McL. Gordon

R. M. Gordon

C. J. Hackett

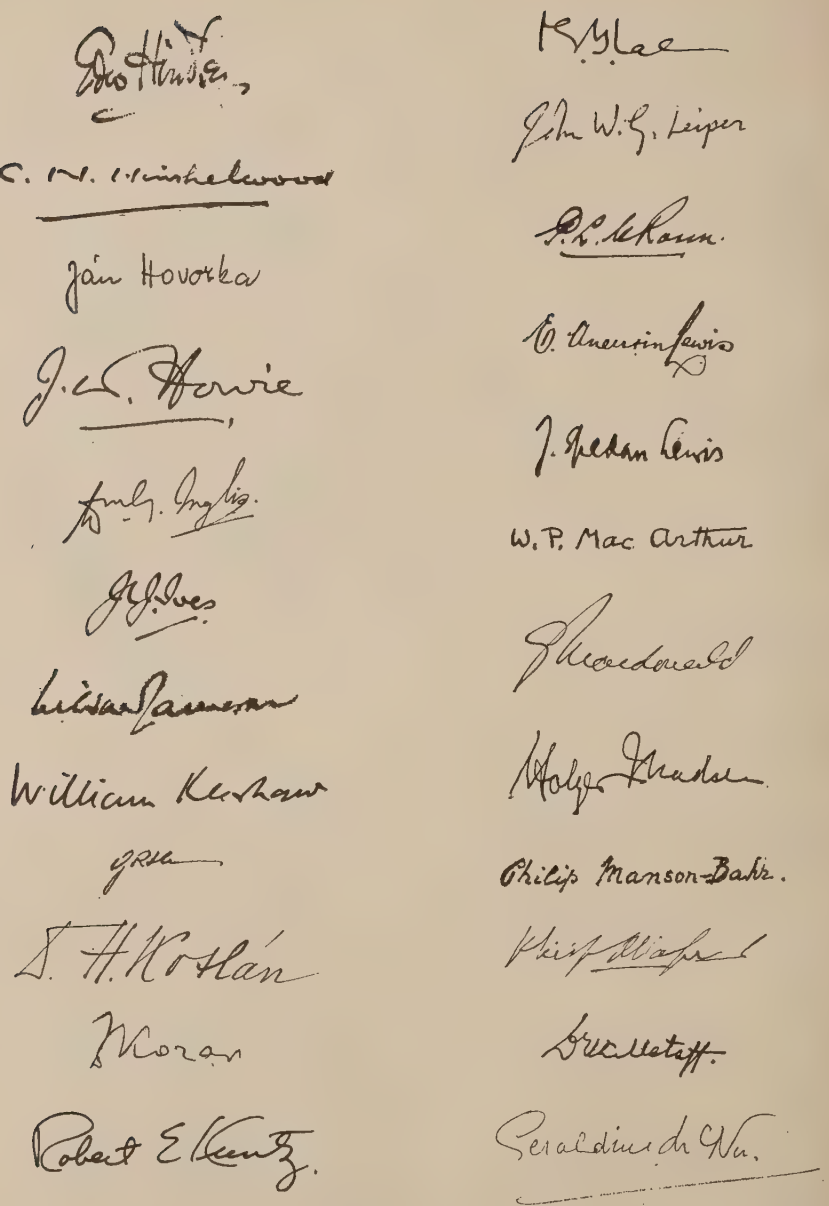
Frank Hawking

A. J. Hesse

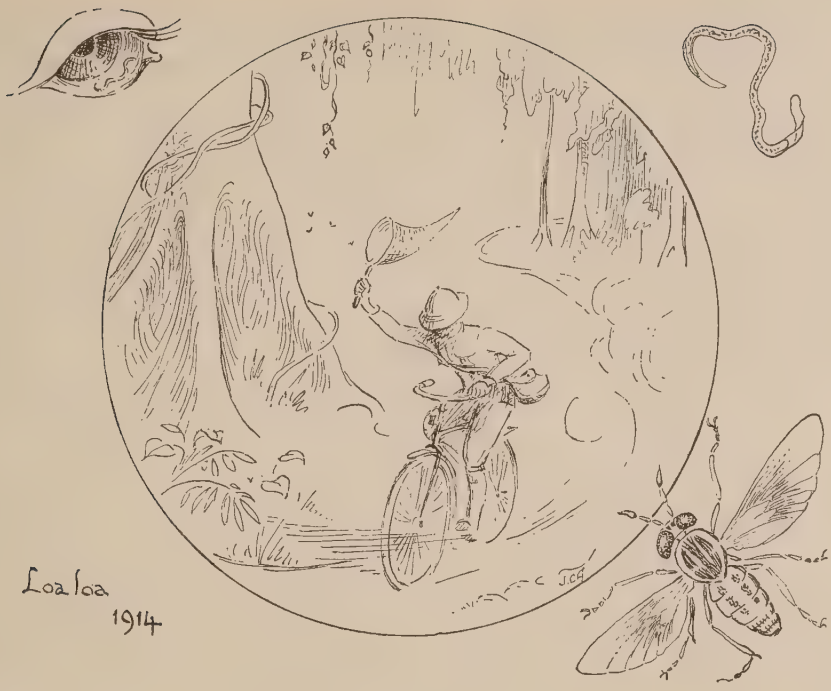
A. Bradford Hill.

First Column : Mary T. Franklin ; Henri Galliard ; P. C. C. Garnham ; Joyce Gear ; V. J. Glanville ; J. B. Goodey.

Second Column : Hugh McL. Gordon ; R. M. Gordon ; C. J. Hackett ; Frank Hawking ; A. J. Hesse ; A. Bradford Hill.



First Column: Edw. Hindle; C. Hinshelwood; Jan Hovarka; J. W. Howie; Wm. G. Inglis; J. J. Ives; Wilson Jameson; William Kershaw; G. R. Kloss; D. H. Kotlan; Z. Kozar; Robert E. Kuntz;
Second Column: M. B. Lal; John W. G. Leiper; P. L. LeRoux; E. Aneurin Lewis; J. Spedan Lewis; W. P. MacArthur; G. Macdonald; Holger Madsen; Philip Manson-Bahr; Philip Maplestone; C. Matoff; Geraldine McNee.



Henry Edmund Meleney

Alan Mozley.

Betty June Myers

J. N. Oldham.

Orloff I. W.

R. J. Ortlepp.

Ivan Parnell.

F. R. N. Pester.

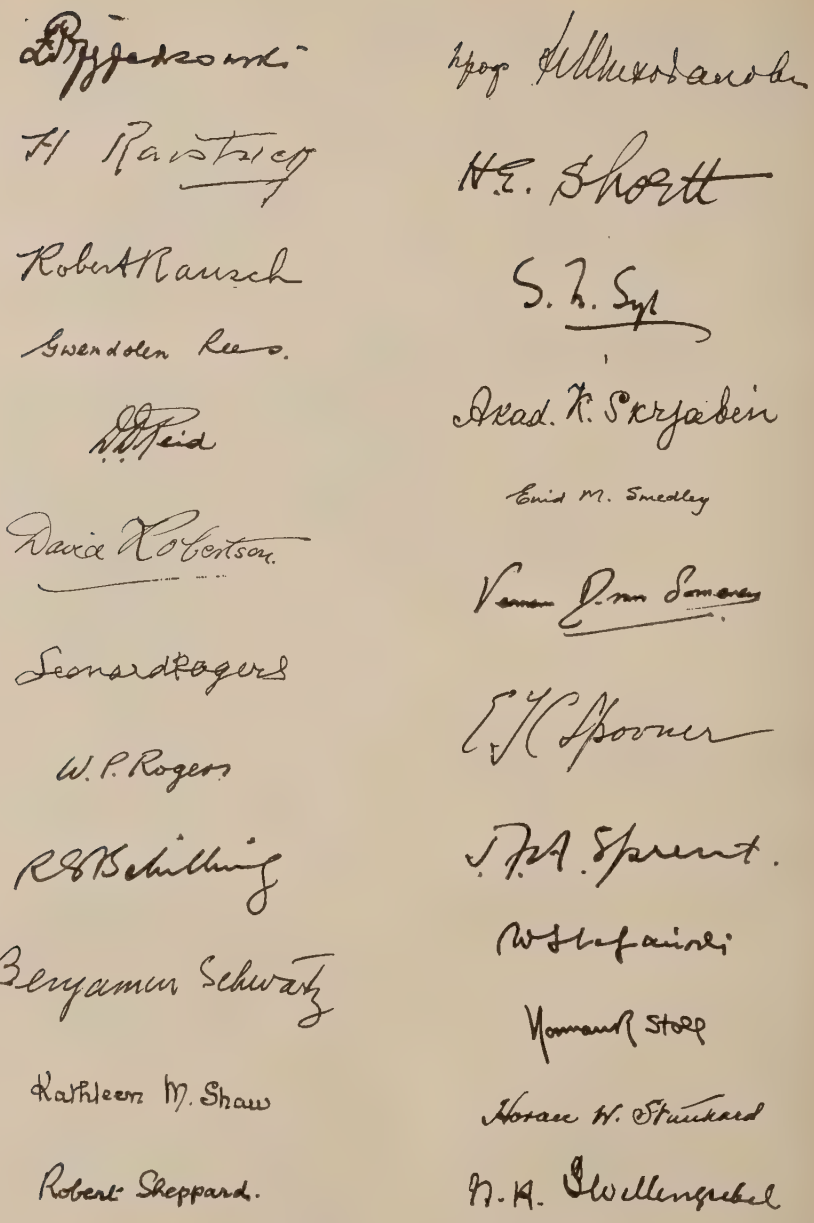
B. G. Peters.

B. S. Platt

L. G. Ponsford

S. Prudhoe

First Column : Henry Edmund Meleney ; Alan Mozley ; Betty June Myers ; J. N. Oldham ; I. W. Orloff ; R. J. Ortlepp.
Second Column : Ivan Parnell ; F. R. N. Pester ; B. G. Peters ; B. S. Platt ; L. G. Ponsford ; S. Prudhoe.



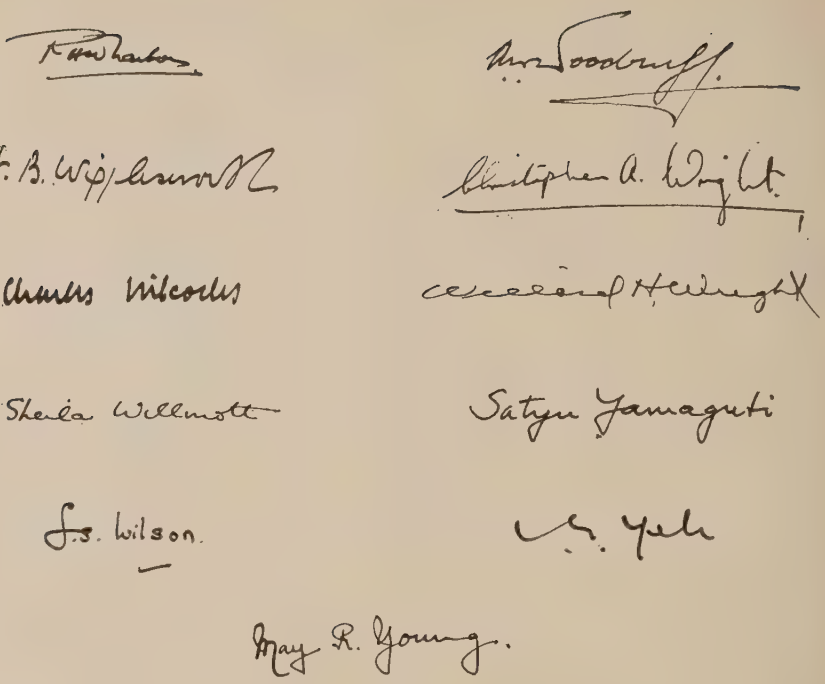
First Column : Z. Przyjalkowski ; H. Raistrick ; Robert Rausch ; Gwendolen Rees ; D. D. Reid ; David Robertson ; Leonard Rogers ; W. P. Rogers ; R. S. F. Schilling ; Benjamin Schwartz ; Kathleen M. Shaw ; Robert Sheppard.

Second Column : N. P. Shikhobalova ; H. E. Shortt ; S. N. Singh ; K. Skrjabin ; Enid M. Smedley ; Vernon D. van Someren ; E. T. C. Spooner ; J. F. A. Sprent ; W. Stefański ; Norman R. Stoll ; Horace W. Stunkard ; N. H. Swellengrebel.

T. Symington.
W. H. Taliaferro
E. L. Taylor
G. S. Thapar
G. Theiler.
Lauro Travassos.

M. Turner.
G. M. Vevers
Hans Vogel
W. S. Walton
J. M. Watson.
Frank Ware.

First Column : T. Symington ; W. H. Taliaferro ; E. L. Taylor ; G. S. Thapar ; G. Theiler ; Lauro Travassos.
Second Column : M. Turner ; G. M. Vevers ; Hans Vogel ; W. S. Walton ; J. M. Watson ; Frank Ware.



First Column : R. H. Wharton ; V. B. Wigglesworth ; Charles Wilcocks ; Sheila Willmott ; G. S. Wilson.
Second Column : A. W. Woodruff ; Christopher A. Wright ; Willard H. Wright ; Satyu Yamaguti ; L.-S. Yeh ; May R. Young.

Citation at the ceremony of conferring the degree of LL.D. on R. T. Leiper by Lord Boyd-Orr Chancellor, Glasgow University, 21st June, 1955

One of the greatest boons in life is to have the power to see ourselves as other see us. It is a sobering thought that as a result of spending some fifty years in the study of tropical medicine, Professor Leiper may know precisely what helminths mean by a worm's eye view of humanity. The philosophic consequences of such enlightenment may well shatter a man's belief in his own lofty status in the animal kingdom; and perhaps the new sense of perspective thus created accounts for Professor Leiper's confession in "Who's who"—that his sole recreation is form-filling.

Our admiration for Professor Leiper's assiduity must increase still further when we remember that, in polite society, parasites are not mentioned except by politicians in search of invective. This may help to account for the restlessness of British parasitologists, though it must be admitted that the scientist is influenced by the searching query:

What do they know of helminths
Who only England know?

Be that as it may, it is certainly true that for half a century Professor Leiper's expert knowledge has been in demand all over the world. On research missions and surveys with important bearings on health and agriculture, he has boxed the compass in his travels between the Gold Coast and Nigeria, China and South America, Egypt and Uganda. His outstanding ability as a field worker, an investigator and an author were recognised when he was still a young man, and in 1941 he was created a Companion of the Order of St. Michael and St. George.

Professor Leiper was educated at Warwick School, but since he is of Ayrshire stock it is scarcely surprising that Glasgow University

Helminthological Research Expeditions undertaken by Professor Leiper from 1905 to 1938

"What do they know of helminths
Who only England know?" *Stanley Alstead.*

shares with Birmingham the honour of claiming him as an alumnus. His degrees in science and in medicine were conferred by this University; he is a Fellow of the Royal Society; and his negotiable assets include three of the most coveted Gold Medals awarded for distinguished work in medical science. Professor Leiper's tenure of the Chair of Helminthology in the University of London contributed substantially to the eminent position enjoyed by the London School of Tropical Medicine; and he now continues to explore new developments in his strange and complex subject as Director of the Commonwealth Bureau of Helminthology at St. Albans.

The University of Glasgow has noted with pride Robert Leiper's achievements in a long life devoted to extending the frontiers of science and medicine. I now ask you, Sir, to confer on him the highest honour which his Alma Mater has the power to bestow, and she will rejoice to see her son in the kaleidoscopic splendour of his triple doctorate.

Stanley Alstead

Leiperigonus Bureaui

Aurevoir, 1958 *nec* **Adieu***

Said *Ann Kylostoma* "Oh, *Me Lord O'Gyne*
Shall *I cosy Ella* while you write a speech
For although you're *incognita*
You would make a smashing speaker
And we must pay homage to Professor R. T. Leiper."
Said *me Lord O'Gyne* "You may cosy or *neglect 'er*
But I'll *stick to Dora* because she's such a peach.
Though R.T.L.'s retiring he doesn't want a fuss
So instead of one speech only
We will have a line from each."
Although he's still resentful
Bill Hart's ire's quickly spent
Though he's now called *Schistosoma*
It's the same worm which is meant.
So he made his contribution
And this is how it went :
"Had it not been for R.T.L.
Our secret we'd have kept
Of how we bored into the snail
And out again we crept
To penetrate the skin of man
And get into his vein
Where we lay lots and lots of eggs
Which pass outside again."
Just then a wail of anguish came—
"*Alas—Oh gone—Oh poor us* !
Oh what has *Alli sat in* now ?"
Cried dear old *Sister Caulus.*
Said *Stizo* "(Come now) *steady on*
It's R.T.L. we're fêting,
We must not let *the lazier* ones
Escape their tribute making.
Do you think *Orrie Batid might*
Add something to our rhyming ?"
He said "I've got *mon niece 'ere* too
How's that for perfect timing ?

* An Ode to Professor R. T. Leiper from some "miscellaneous helminths" on the occasion of his retirement from the Commonwealth Bureau of Helminthology.

I seen yer in the early days
Researching the World over,
I only hope when you retire
You'll find yourself in clover !"
A cry then went up from his staff
"He's *strong while us* is weak
He turns his abstracts out so fast
We've hardly time to speak."
So please accept this typewriter,
An Olivetti Lettera,
E'en though your writing's VERY clear
Typewriting is much bettera !
We hope that you will write a book,
Memoirs would make good reading
Though they might land you in the Courts
To do some special pleading !
So, Robert Thomson Leiper, *Oh Creator* of H.A.
We wish you many happy years' retiral from the fray !

With due apologies and acknowledgments to :
Ancylostoma
Meloidogyne incognita
Icosiella neglecta
Stictodora
Bilharzia
Allassogonoporus
Allisatin
Cystocaulus ocreatus
Stizostedion
Thelazia
Oribatid mites
Moniezia
Eisenia
Strongylus
H.A.=Helminthological Abstracts

SHEILA WILLMOTT

Robert Thomson Leiper

Robert Thomson Leiper has been more closely associated with the School of Tropical Medicine in London than any one else. It is 55 years since he joined the staff in its early days, in 1905, some six years after its foundation, so it is difficult to realize now from his appearance, or alertness, that he has completed more than his half-century. During all this long time he has constituted himself guide, philosopher and friend of this venture and has, almost more than anyone else, been the pioneer of its destinies. Now, in the 38th year of the Journal of Helminthology, which is his child, he has more claim to be adjudged the "Father of Modern Helminthology" than anyone, so it is fair to claim that he is by far the most distinguished in this speciality in the world to-day. He has been associated with the great Masters in the past, with Patrick Manson, Arthur Looss, F. Fülleborn of Hamburg and Wardell Stiles of America, so therefore has a good background. Leiper was reared in helminthology and has had his being in this speciality which in many ways he has made his own and no one has contributed more to the knowledge of its tropical aspects. Long before his advent to the School, as a shy youth, Leiper had been devoted to helminthology at the Millport Marine Station while a medical student at Glasgow, had been singled-out by a friend of Patrick Manson's, and had drawn attention to his qualities. Just think of his great discoveries. It is a most impressive list :—The unravelling of the life-history of *Loa loa* in 1913 ; the classical descriptions of many new helminths—*Ternidens deminutus* (1913), *Physaloptera mordens*, *Lagochilascaris minor* and others ; then the almost fabulous story of the developmental cycle of *Schistosoma haematobium* and *S. mansoni* in 1915 and the differentiation of the adult forms, all of which contributed so much to the progress of the Great War. It is therefore true to say that it was Leiper who put Tropical Helminthology on the map. In addition to these victories he has made great contributions to Veterinary and Agricultural Helminthology by founding the Commonwealth Bureau of Helminthology as well as the Institute of Agricultural Parasitology at St. Albans. He has also rendered inestimable services in issuing his Catalogue of Periodicals of Medicine and Allied Sciences in British Libraries and furthermore his life-long task has been the recording of the literature of helminthology.

Now as to the man. No one can deny that Leiper is a character hammered out from his Scottish ancestry. He is kind, charming and helpful to the worthy, but withering to the insincere or make-believe. He has a distinctive smile which he varies to suit the occasion and he is a critic of no mean order, but his criticisms are tempered by his high sense of humour. Nothing pleases him so much as a friendly dig at amateur helminthologists. These little outbursts are very welcome and cannot be resented. But he is more than a critic, he is a diplomat and strategist of no mean order. To watch him at a committee is an education in itself, and to witness him concentrate his forces so as to annihilate all opposition is a lesson. But whatever feelings one may harbour about his dagger-like thrusts there is one man he has never criticized by word or deed and that man was his Master, Patrick Manson.

Philip Manson-Bahr

Professor Leiper's Contribution to Agricultural Helminthology

Robert Thomson Leiper, C.M.G., J.P., M.D., D.Sc., LL.D., F.R.C.P., F.R.S., Professor Emeritus of Helminthology in the University of London, Director for many years of the Department of Parasitology, London School of Hygiene and Tropical Medicine, of the Institute of Agricultural Parasitology, and of the Commonwealth Bureau of Helminthology, was born in Kilmarnock, Ayrshire, on the 17th April, 1881, and educated at Warwick School, Mason University College Birmingham and Glasgow University; he graduated in medicine in Glasgow in 1904.

His interest in helminthology, begun and encouraged whilst an undergraduate by his cousin, Professor Gemmill, continued (thanks to a Carnegie Research Scholarship in Biology) with a study of the Turbellaria of the Scottish National Antarctic Expedition. However, his stay in Glasgow was short-lived, because soon after (in 1905) he and another young medical graduate, C. M. Wenyon, were appointed by Sir Patrick Manson to the newly created positions of Helminthologist and Protozoologist, respectively, at the London School of Tropical Medicine which had been opened in 1899 at the Royal Albert Docks, London, E.

Leiper's primary duties at the School were concerned with teaching helminthology to medical graduates reading for the London or Cambridge Diploma in Tropical Medicine. This was formal class-room teaching which dealt with human disease-carrying organisms in the tropics. After the first World War, this was supplemented by similar teaching for the Dipoma of Public Health. His influence on health problems in the tropics—and to a lesser degree in temperate climates—was considerable, particularly when one realizes that there was practically no instruction in parasitology in the medical schools after the preliminary very sketchy course in zoology given at the beginning of the students' undergraduate career. Misconceptions about parasitology were legion. Leiper did much to redress the balance and helped to make the London School of Tropical Medicine one of the great teaching institutions of the world. Students came from all tropical countries for courses which at first lasted some three months, but later were extended to five; during the second World War these were replaced by an almost continuous series of short courses of two weeks, attended by nearly 2,000 medical officers from the allied Forces.

The peace time teaching was on a "block" system, each of the four major subjects (helminthology, protozoology, entomology, and bacteriology-mycology) lasting for about four weeks and occupying the major part of each working day. Much of the work was done in the laboratory. Lectures on clinical aspects of tropical diseases were given by visiting physicians in the late afternoon.

This technique of block-teaching left much time for research. This fitted with Leiper's own inclinations and under the influence of Sir Partick Manson—that same influence which encouraged Ross to prove the rôle of the mosquito in malaria—he commenced his investigations in tropical helminthology, investigations which in time touched upon almost every parasitic worm of importance to man.

Undoubtedly, the most outstanding of Leiper's many contributions was concerned with the Schistosomes or Bilharzia worms, several species of which live in the bloodstream of man and other animals and are estimated to infect about one-twentieth of the human race—over a hundred million persons—and cause one of the major disease complexes of mankind in the tropics.

Leiper's work, of course, covered a much wider range than the Bilharzia worms. He investigated the life history of the Guinea worm and discovered the elusive male of that species. He found that *Loa loa* of West Africa was carried by certain species of Tabanid flies. He described several new species of intestinal nematodes of man. He led an extensive investigation into British Guiana to study the pathology and epidemiology of Filariasis. He infected himself with the Fish tapeworm to determine its potential pathology.

The end of the first World War saw an increasing interest in parasitology. In the early days of medicine, it had occupied a place of considerable importance but bacteriology had been gradually displacing it. However, the institution of the degree of Doctor of Philosophy, and Leiper's reputation, caused a resuscitation of the subject and the development of a postgraduate school in helminthology at the London School. Leiper had a wide concept of how the subject should be studied and he insisted on the fundamental idea that it is a single entity whether one's primary interest is in the parasites of man, of domestic or wild animals, or even of plants, always returning from his overseas expeditions with a varied collection from the local fauna.

He was one of the earliest of the comparative helminthologists. He recognized the fact that all animals, not merely domestic ones, had these parasites and that an invaluable source of research material lay in Zoological Gardens. Immediately after the war he became—in addition to his professorial appointment—Director of the Prosectorium of the Zoological Gardens in London. Although he held this position for only three years, he and his assistants attended the Prosectorium for at least a decade thereafter in a daily search for new and interesting parasites which could be used for teaching or research. Among other investigations arising from this material, Leiper recorded the parasites of the almost newly discovered Okapi.

His activities were not confined to wild animals or birds in captivity, however, and the part he played in the celebrated investigation of Grouse Disease in Scotland is well known. But it was in farm animals that parasites were most important. The Ministry of Agriculture became

interested in 1921 and Leiper was among the first to receive a research grant to investigate the parasites of farm animals. Various biologists were added to his staff in London and active work began on the parasites of sheep, cattle, pigs and horses, as well as on the material collected at the London Zoo.

So many researches were undertaken that Leiper founded and published the Journal of Helminthology in order to find a speedy outlet for the papers resulting from this work. The first few volumes were devoted exclusively to articles by his relatively small staff—a permanent tribute to the enthusiasm which his direction inspired. That staff is now widely dispersed. Some are dead, others occupy Chairs of Parasitology in many of the Commonwealth countries where second or even third generations of parasitologists are being raised in the Leiper tradition of reading and thinking and experimenting to understand the complex life of the parasitic worms.

In 1924, a capital grant was obtained from the Development Fund to establish a field station for this work. A field station must of necessity be located in the country and Leiper searched far and wide throughout the Home Counties for suitable premises—finally selecting a derelict farm of some thirty acres, near St. Albans, which subsequently became known as the Institute of Agricultural Parasitology.

Most people would have pronounced Winches Farm entirely unsuitable—a farm house in disrepair, tumble-down farm buildings, some in imminent danger of collapse, a stock-yard inches deep in mud—all made up a depressing picture. Winches Farm was cheap, however, and funds were limited and Leiper could see through the existing chaos to the possibilities which later were actualized. One of his minor attributes has been a flair for transforming dilapidated buildings ; given a free hand with a jobbing carpenter and a trivial financial grant and, as at Winches, cart sheds and outhouses became fully serviced laboratories and an old stable became a miniature banqueting hall which countless visitors have admired. By such changes as hacking out hideous Victorian fireplaces to reveal the original open hearth beneath, the farm house itself was restored to something like its Sixteenth Century beauty.

The initial research at the Farm was concerned with sheep and goats but it quickly was enlarged to include helminth parasites of insects and plant eelworms—a subject which in the passing of the years has become of increasing agricultural importance but which in the 1920's was still in process of being developed. The Institute persisted for twenty-three years. In 1946—a year after Leiper's retirement as Director—the work on eelworms was transferred to the new Nematology Department at Rothamsted Experimental Station, Harpenden and Winches Farm became the Field Station of the London School of Hygiene and Tropical Medicine. Thus the Institute's work continues to flourish under other names, though its comprehensive scope is now lacking.

No branch of science can flourish without an adequate literature and because helminthologists were few and widely scattered, references

were often difficult to trace. Leiper was always alive to the bibliographical problems of his subject and in 1923 he published his "Periodicals of Medicine and the Allied Sciences in British Libraries"—a forerunner of the "World List of Periodicals". His knowledge of the literature of his subject is profound; no one is better acquainted with the early writings on helminthology and his library of these works is unsurpassed. An Imperial Agricultural Research Conference was called in 1927 to consider the collection and dissemination of scientific information bearing upon agriculture and as a result eight specialist Imperial Bureaux were created—one of which, now called the Commonwealth Bureau of Helminthology, came under Leiper's direction and which he continued to direct for thirty years.

In 1929, the Bureau came into existence first in a converted byre, later in the old farm house at Winches Farm—with an office at the School in London. The filing and indexing of titles and abstracts began forthwith, leading to the publication of the "Bibliography of Helminthology" for 1930 and 1931. In 1932, this was incorporated in "Helminthological Abstracts", now in its thirtieth year of publication. The abstracts were published in an unusual form, being grouped by journals rather than by subjects. As each volume was deliberately confined to papers published during one calendar year, the issue of the final part of each volume was in consequence always postponed to provide for the inclusion of abstracts of papers which were traced with difficulty.

Leiper had long been associated with the Tropical Disease Bulletin of course, and had developed the ability to condense the essence of an article clearly and concisely to an irreducible minimum of words. Helminthological Abstracts (many of the abstracts being from his own pen) continued the practice, making them the basic research tool for all helminthologists.

Leiper retired in 1958—officially—but he is still hard at work in a room in the White House at St. Albans—the present headquarters of the Bureau. His administrative and organizational duties are completed and he is again free to turn once more to his personal research.

It is difficult to determine what constitutes a great biologist. It is a complex of almost indefinable characteristics. These include a clear but logical imagination, the ability to visualize the future trend of his research, and a boundless energy. Perhaps even more important is the gift of teaching his students how to learn so that in this manner his own work can never be completed and his influence on his subject can never diminish.

Ian Clunies-Ross
Thomas W. M. Cameron
Bernard G. Peters

Other Activities

Much has been written about Professor Leiper's achievements in the scientific sphere yet there is still much to be recorded and in the limited space of a "festschrift" full justice cannot be done to this subject. For example, a list of his scientific publications, numbering over 130, might have been included. It is equally impossible on this occasion to give an adequate account of the many other aspects of his long and brilliant career which in itself would merit a separate volume. The following précis is intended merely to indicate the breadth of his interests during a lifetime of outstanding activity.

He was a Member of the following Scientific Bodies : Royal Society of London, (*Member of Council, Member of the Tropical Diseases Committee and Abstracting Services Consultative Committee*) ; Royal Society of Medicine, (*Vice President of Council and President of the Sections of Comparative Medicine and Tropical Diseases and Parasitology*) ; Royal Society of Tropical Medicine and Hygiene, (*Councillor*) ; British Medical Association, (*Vice President of the Tropical Medicine Section, Centenary Year, and Vice Chairman of the Dominions Committee*) ; West Indian Medical Conference in British Guiana, (*Vice President*) ; Zoological Society of London, (*Director of the Prosectorial Committee*) ; League of Nations Schistosomiasis Conference in Geneva, (*Chairman*) ; Italian Society of Tropical Medicine Conference, (*Hon. Vice President*) ; Association of Applied Biologists, (*Treasurer and Member of Council*) ; British Empire Cancer Campaign, (*Member of Grand Council and Scientific Advisory Committee*) ; Lister Institute, (*Member*) ; University of London, (*Boards of Studies in Hygiene, Agriculture, Zoology, Pathology and Estate Management*).

He received Honorary Membership of : the National Veterinary Association ; National Institute of Sciences of India, (*Hon. Fellow*) ; American Society of Parasitologists ; Helminthological Society of Washington ; Société Pathologique Exotique, Paris ; Société Belge de Médecine Tropicale and the Accademia di Medicina, Rome.

He was a Member of the Egyptian Government Survey in Uganda 1907 ; he was Consultant Parasitologist and temporary Lieut.-Col. R.A.M.C., Bilharzia Mission in Egypt (1915-1916).

He served on two important Government Bodies—Colonial Office, (*Member of the Advisory Medical Committee and the Colonial Veterinary Services Committee*) ; Agricultural Research Council, (*Member of the Committees on Animal and Plant Helminths, Swine Fever and Similar Diseases, Animal Diseases and Animal Nutrition*).

His services as External Examiner in helminthology and related subjects were much in demand from the Royal College of Veterinary Surgeons (*Veterinary Parasitology*) and the Conjoint Board (*Parasitology*). He was examiner for helminthological theses for higher degrees in the

Universities of London, Cambridge, Bristol, Birmingham, Reading, Liverpool, Aberdeen, Edinburgh, Dublin, Galway, Wales, Melbourne, McGill (Montreal), Cairo, Allahabad, Lahore, Lucknow, Madras, Osmania (Hyderabad) and Panjab.

He was Assessor for Medical Research Scholarships of the Grocers' Company.

No précis of his career would be complete without some mention of Professor Leiper's association with the London School of Hygiene and Tropical Medicine (of which he was at one time Acting Dean) and its predecessors at Endsleigh Gardens and the Royal Albert Docks. Suffice it to say that his energy and far-seeing administrative vision have played a prominent part both in the evolution and transitional episodes of the School throughout its celebrated existence. To him also must be attributed much credit for the world-wide honoured position accorded to the School as a scientific centre for teaching and research ; his able Directorship of the Department of Parasitology did much to enhance this reputation.

During his long residence in Hertfordshire, Professor Leiper took a keen interest in local affairs ; he served on local government bodies such as the St. Albans Rural District Council of which he was Vice Chairman ; St. Albans Joint Hospital Board, (*Member*) ; Wheathampstead Parish Council, (*Chairman*). He has been a Justice of the Peace for Hertfordshire for many years.

Professor Leiper's affinity for the rural environment is exemplified by his enjoyment of countryside rambling and by his ownership of three farms, two of which he farmed in Scotland and one in Hertfordshire, undertakings which illustrate his boundless and characteristic energy ; his aptitude for "converting" old farm houses, almost single-handed, could have earned for him the reputation of being the originator of the "do-it-yourself" cult. To furnish these buildings he would attend the local auction sales where his keen eye for a good bargain or a useful piece of equipment served him well.

As a scholar and writer and speaker, Professor Leiper has ample cause for pride in personal achievement. One of his most treasured realisations—the ambition of every Scot—was to propose the "Immortal Memory" for which he characteristically took as his theme—not Robert Burns' love poems—but his many Natural History observations.

In the year 1958 he retired from the Directorship of the Commonwealth Bureau of Helminthology, at the early age of 77, but since then he has continued as a voluntary worker at the Bureau in St. Albans.

His recent re-appointment, nearing his 80th birthday, as Consultant Helminthologist to the Bureau in an active capacity, will come as no surprise and will give much pleasure to those who are aware of his indefatigable zest for work and for promoting the interests of helminthology.

May he enjoy this "recreation" *ad multos annos !*

J. J. C. Buckley (*Editor*).
L. M. Russell (*Assistant Editor*)

hotograph by the "Herts Advertiser"

rofessor Leiper receiving from Sir William MacArthur a presentation copy of the *Festschrift* (*Journal of Helminthology*) the London School of Hygiene and Tropical Medicine on the occasion of his 80th birthday, 17th April, 1961.

hotograph by Mr. L. F. Khalil

resentation of the Bernhard-Nocht Medal. *Left to right* : Sir Wilson Jameson, Dr. Elsie Toms (Mayor of St. Albans), rotessor R. T. Leiper, Mr. Elmar Weindel, (Attaché to the Embassy of the Federal Republic of Germany), Professor an G. Baer (Switzerland), Professor T. W. M. Cameron (Canada), Professor G. Macdonald (Acting-Dean, London School of Hygiene and Tropical Medicine).

TROPENINSTITUT
BERNHARD-NOCHT-STRASSE 74
HAMBURG 4
TELEFON 31 13 21

April 12, 1961

Dear Dr. Leiper,

I have great pleasure in informing you that the staff members of the Hamburg Tropeninstitut have agreed upon unanimously to award to you the

Bernhard-Nocht-Medaille

for your outstanding merits in the field of Tropical Medicine.

We may ask you to accept this scientific distinction awarded to you on the occasion of your 80th birthday as a token of our veneration and gratitude.

With best wishes

Yours sincerely

Prof.Dr.Nauck
Director

Contents

	PAGES
Host Reactions in Young Birds to Naturally Occurring Superinfestations with *Porrocaecum ensicaudatum* : Jean G. Baer	1—4
On a New Parasitic Nematode *Trichosomoides nasalis* n.sp., from the Nasal Cavities of *Epimys norvegicus* : and Considerations on the Family Trichosomoididae Yorke and Maplestone, 1926 : E. Biocca and A. Aurizi ...	5—8
The Laboratory Diagnosis of Bilharziasis : W. K. Blackie	9—16
Anomalous Results from an Experimental Infection of Man with *Brugia malayi* (Brug, 1927) : J. J. C. Buckley and R. H. Wharton	17—24
On a Phylogenetic Classification of the Family Trichostrongylidae Leiper of Marsupials, Insectivores and Edentates : T. W. M. Cameron and Betty J. Myers	25—34
Recent Observations on Helminthiasis in some British Game Birds : Phyllis A. Clapham	35—40
On the Early Stages of *Fasciola hepatica* Penetrating into the Liver of an Experimental Host, the Mouse : a Histological Picture : Ben Dawes	41—52
On the Morphology and Life History of *Fasciola nyanzae* Leiper, 1910 from the Hippopotamus : J. A. Dinnik and N. N. Dinnik	53—62
Estimation of Field Populations of Cyst-forming Nematodes of the Genus *Heterodera* : D. W. Fenwick ...	63—76
Records of Mortality from Helminth Diseases in England and Wales up to the Year 1880 : A. E. Fountain ...	77—84
A British Root-knot Nematode, *Meloidogyne artiellia* n.sp.: Mary T. Franklin	85—92
Hormones Corticotropes et Cortico-surrénales dans les Infections à Helminthes : Henri Galliard	93—100
The Nature of the Spear Guiding Apparatus in Dorylaimidae : Basil J. Goodey	101—106

Passage of Eggs by Hosts Infected with *Schistosoma haematobium* : Robert E. Kuntz ... ... 107—116

Some Problems in Bilharziasis in Africa and the Adjoining Countries : P. L. LeRoux ... ... 117—126

Studies on Parasites of the Grey Squirrel (*Sciurus carolinensis* Gmelin) from South Eastern England. I. Helminth Parasites : J. N. Oldham ... ... 127—130

On Two Rictularias and a Filariid from South African Wild Carnivores : R. J. Ortlepp ... ... 131—140

Heterodera rostochiensis Population Density in Relation to Potato Growth : B. G. Peters ... ... 141—150

The Physiology of Hatching of Eggs of *Ascaridia galli* : W. P. Rogers ... ... 151—156

On a New Species of the Genus *Chevreuxia* Seurat, 1918 from India : S. N. Singh and S. M. Ali ... ... 157—160

Investigations on the Anti-bacterial Properties of Infective Larvae of Some Parasitic Nematodes : W. Stefański and Z. Przyjalkowski ... ... 161—168

Favored RLE for Axenic Culture of *Neoaplectana glaseri* : Norman R. Stoll ... ... 169—174

Acquired Immunity and Helminthic Disease : E. L. Taylor ... ... 175—178

The Life History of *Olveria indica*, an Amphistome Parasite from the Rumen of Indian Cattle : Gobind Singh Thapar ... ... 179—186

Sur un Curieux Nématode, *Robertia leiperi* gen. et sp. nov., Parasite de l'Intestin Postérieur de Diplopode : L. Travassos et G. R. Kloss ... ... 187—190

Über eine unsegmentierte, eingeschlechtliche *Taenia saginata* : H. Vogel ... ... 191—198

The Breeding of *Bulinus truncatus* in Total Darkness : J. M. Watson and A. S. Al-Ali ... ... 199—204

A Study of the Gastrodiscinae Monticelli, 1892 (Gastrodiscidae : Paramphistomoidae) : Sheila Willmott and F. R. N. Pester ... ... 205—216

Studies on the Helminth Fauna of Japan. Part 57. Nematodes of Fishes, III : Satyu Yamaguti ... 217—228

Index of New Names 229

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 1–4.

Host Reactions in Young Birds to Naturally Occurring Superinfestations with *Porrocaecum ensicaudatum*

By Jean G. Baer, F.M.L.S.
Institut de Zoologie, Université de Neuchâtel

The young of all nest dwelling birds are fed continuously by their parents. It is the latter therefore who choose the food which is mostly composed of that which the adult birds feed on. Under these circumstances it is therefore not surprising that fledglings when still in the nest are found to harbour helminths of which the larval forms were contained in the food*.

It is possible, at times, to find enormous numbers of worms in young nestlings whereas the adult birds only harbour one or two specimens. We have reported (1956) having found the crop of young Fulmars taken on the nest to contain hundreds of specimens of *Stegophorus stellae-polaris* (Parona) whereas this species is not very common in adult birds. It seems that such cases must not be uncommon but usually escape the notice of helminthologists working in the field; nor has the mechanism by which young birds rid themselves of such an excess of parasites been given any particular attention. For helminths that migrate at one stage or another through the host's tissues, it seems plausible that some sort of immunological reaction arises.

It is for this reason that we think it might be interesting to report a series of observations made throughout the year on a population of blackbirds that feeds in the immediate vicinity of our Institute. We have previously reported (1960) the presence of *Euamphimerus pancreaticus* Baer in the pancreatic ducts of these birds and have drawn attention to the fact that the parent birds feed their young on very small fishes, which would account for the source of this infestation. Unfortunately further work on this subject has been handicapped by bad meteorological conditions and an epizootic amongst the fishes of the lake of Neuchâtel.

* An exception to this rule is found in young cuckoos which apparently never harbour their foster-parents' tapeworms.

Of the sixty blackbirds that were examined for parasites, about 25% were infested with the ascarid *Porrocaecum ensicaudatum* (Zeder, 1800). Heavy infestations with adult worms were rare except in a single case of a young bird from this year's first brood, shot on May 16th, 1960. Not only did the gut contain more than one hundred adult worms, but all stages of development were found within the intestinal mucosa.

Several years previously while examining earthworms (*Allolobophora chlorotica* Sav.) collected in a nearby wood we found, in loam covered with blackbird droppings, one particular population of earthworms which was heavily parasitized by larval nematodes occurring within the ventral blood vessel. About 60% of the earthworms were parasitized. When fed to young blackbirds and to ducklings, the nematode larvae were found within 48 hours beneath the horny layer of the gizzard. Unfortunately this experiment could not be continued.

Osche (1955) has recently described the third larval stage of *Porrocaecum ensicaudatum* from the ventral blood vessel of *Lumbricus herculeus* Sav. from Erlangen (Germany). His very accurate description draws attention to the fact that the gut of these larvae is filled with blood and that the intestinal cells are densely packed with fine, dark brown granules which appear to be derived from digested blood. The whole gut is distinctly visible on this account especially when the earthworm is examined by transmitted light (Fig. 1). On all sections of the third larval stage the intestinal cells are densely black (Figs. 2 and 3).

The third larval moult appears after the larvae have burrowed beneath the horny layer of the gizzard (Fig. 4) and it is usually assumed that the fourth stage larvae pass into the gut lumen and later become adult.

On several occasions we have found such fourth stage larvae measuring about 10–11 mm. in length and recognizable by the remains of digested blood within the intestinal epithelium, at different levels of the intestine within the mucosa. Usually, the anterior extremity of the larva pierces the surface of the mucosa and the entire worm may be easily removed with fine forceps. A section passing through such a larva *in situ* (Fig. 5) distinctly shows the pigment granules in the gut wall which are responsible for the black, hair-like thread that appears against the pink background of the

bird's intestine. The section also shows the presence of a distinct host reaction surrounding the larva and consisting chiefly of leucocytes and eosinophilic cells. This reaction probably accounts for the ease with which the larvae can be removed from the mucosa.

The question arises as to whether the presence of the fourth larval stage within the intestinal mucosa of the host is a normal phase of the life cycle or whether it represents a reaction of the host to successive infestations. We are inclined to adopt the latter alternative in view of our findings in the young blackbird, mentioned above, in which fibrous tumours occurred on the peritoneal surface of the intestine. The presence of such tumours has never been recorded in any of our blackbirds, either in adult or in young birds. Nor have we ever come across a bird so heavily infested as that described in this paper.

We have been fortunate in finding in our sectioned material evidence of that which we interpret as being the cause of these tumours, namely, a host reaction against an exceptionally heavy infestation with larval worms.

When a section of a tumour is examined under the microscope, the centre is formed by a cavity, whose walls are lined by a syncytium of giant cells ; outside this lies the fibrous connective tissue reaction (Fig. 7). Within the cavity the fifth larval stage is found, partly surrounded by the fourth moult. This reaction occurs every time that a fourth stage larva moults within the mucosa and it might quite possibly be elicited by a direct effect of the moulting fluid on the host's tissues, judging from the purely localized reaction. In Fig. 6 is seen an early stage of this process and in Fig. 8 the ultimate stage where the worm has been destroyed and the tissues are beginning to be restored.

There seems to be no doubt whatever that we are dealing here with a self-defence reaction of a host towards a series of successive infestations with larvae of *P. ensicaudatum*. An indication that some form of an immunological reaction of a more general type also occurs, is found in the presence of the fourth larval stages within the mucosa all along the intestine and the fact that these larvae moult while still in the tissues of the host. There thus appears to exist a general inhibitory mechanism affecting normal larval development that finally leads to the destruction by phagocytes of the fifth larval instars.

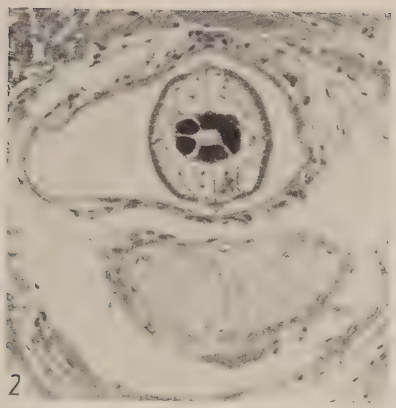
This kind of defensive reaction in a normal host does not seem to have been described before but probably only occurs in young birds that have been heavily infested by their parents. It shows how a juvenile host is able to rid itself of an excess of worms even though it does so at some expense to its organism. The young blackbird mentioned above was looking very sorry for itself, its feathers were ruffled and it had trouble keeping its balance when hopping on the ground.

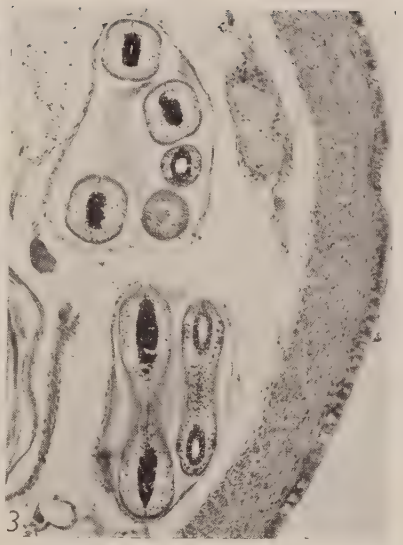
References

Baer, Jean G., 1956.—"Parasitic helminths collected in West Greenland." *Medd. Grønland*, **124**, 55 pp. (W.L. 12862).

———, 1960.—"Une nouvelle espèce de Trématode, *Euamphimerus pancreaticus* n.sp. (Opisthorchiidae)." *Bull. Soc. neuchâtel. Sc. nat.*, **83**, 63–70. (W.L. 5299).

Osche, G., 1955.—"Ueber Entwicklung, Zwischenwirt und Bau von *Porrocaecum talpae*, *Porrocaecum ensicaudatum* und *Habronema mansioni* (Nematoda)." *Z. Parasitenk.*, **17**, 144–164. (W.L. 23536b).





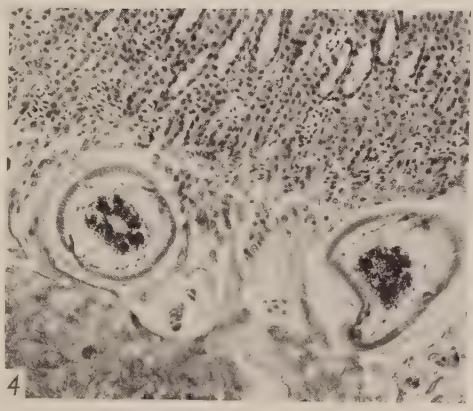
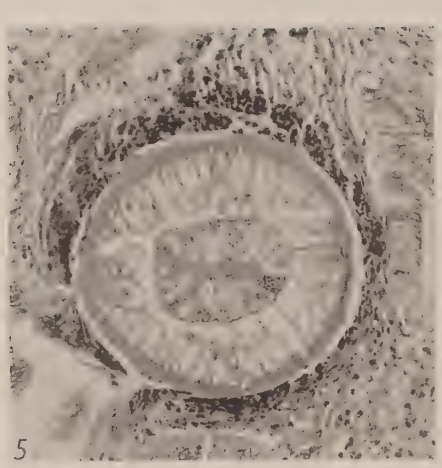
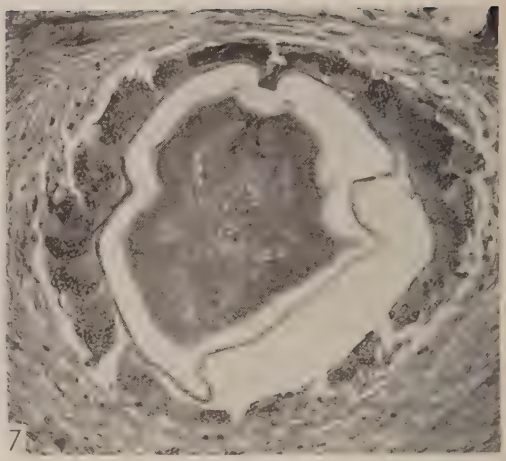
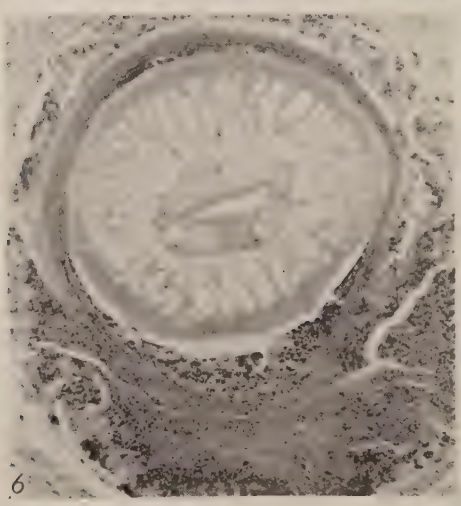


Fig. 1.—Earthworm with gut removed, showing the third larval stage (*P. ensicaudatum*) inside the ventral blood vessel. Fig. 2.—Transverse section of an earthworm showing a larva with intestinal cells densely packed with pigment granules lying within the ventral vessel immediately above the longitudinal nerve chain. Fig. 3.—Transverse section of an earthworm showing several larvae coiled within the ventral vessel. Fig. 4.—Section of the gizzard wall of a young blackbird infected experimentally 48 hours previously with earthworms. The horny layer is visible in the bottom left hand corner and the third moult is clearly seen surrounding the fourth larval stage.







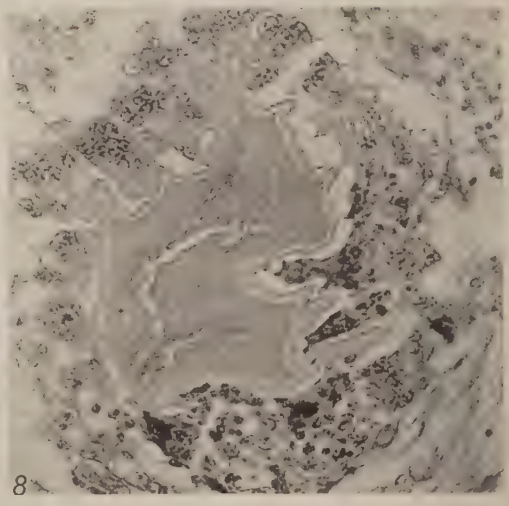


Fig. 5.—Section of a fourth stage larva (*P. ensicaudatum*) within the intestinal mucosa of the host. The pigment granules within the gut cells of the larva are much less numerous and a mild host reaction is seen around the larva. Fig. 6.—Section of a fifth stage larva within the intestinal mucosa of the host. The fourth moult is visible in the upper part of the section and a violent host reaction with giant cell formation in the lower part. Fig. 7.—Section of a tumour in the centre of which is a fifth stage larva surrounded by a syncytium of giant cells. Fig. 8.—Section through an ultimate stage where the nematode has been destroyed by the host reaction. Several giant cells are still visible as well as the "loosening up" of the fibrous structure of the tumour.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 5–8.

On a New Parasitic Nematode *Trichosomoides nasalis* n.sp., from the Nasal Cavities of *Epimys norvegicus*: and Considerations on the Family Trichosomoididae Yorke and Maplestone, 1926

By E. Biocca and A. Aurizi
From the Istituto di Parassitologia dell'Università di Roma

In the nasal cavities of nearly 20% of *Epimys norvegicus* captured in the city of Rome were found parasitic nematodes of the genus *Trichosomoides* Raillet, 1895. These parasites were never found during several post mortem examinations of rats captured in the country nor in laboratory bred white rats. It has been possible to obtain experimentally the infection of the nasal cavities of white rats following the ingestion of embryonated eggs of this species, which can be easily differentiated from *Trichosomoides crassicauda* (Bellingham, 1865) Raillet 1895, which is the only known species of the genus up to the present time, (Aurizi, 1958).

For the new species we propose the name *Trichosomoides nasalis* n.sp., on account of its peculiar *habitat.*

Trichosomoides nasalis n.sp.

Measurements of 10 females and 10 males :

Female and male of very different shape ; male much smaller and commonly found in the uterus of the female.

Female : Length 16–28 mm.; maximum width 0·25–0·35 mm. The body enlarges gradually from the head to near the junction of the anterior and medial third of the body, then maintains the same width. Mouth apical, surrounded by minute papillae. Cuticle with well developed transverse striations corresponding to the second half of oesophagus. Vulva near or slightly posterior to the end of the oesophagus with two prominent lips, 2·9–3·4 mm. from the anterior extremity of the body. From the vulva to near the posterior end of the body, especially on the ventral side, there are papilla-like cuticular elevations nearly 10μ in diameter.

Oesophagus : Length 2·8–3·35 mm.; anterior portion 250–300μ long, devoid of cell body, and long posterior portion surrounded by a cell chain.

Ovary nearly 2 mm. long, beginning generally with a short terminal loop in the posterior extremity of the body, followed by a long oviduct and continued by the uterus in which the male can generally be seen. The last part of the uterus and vagina are long and clearly muscular, with eggs disposed in one single row.

Eggs : 0·038–0·048 × 0·065–0·084 mm. containing embryos, lemon shaped, dark golden when in the last part of uterus and vagina ; often opercular plugs on both poles are not clearly seen. Anus terminal or subterminal. The posterior extremity of the body, especially in preserved specimens, is ventrally bent.

Male : Length 1·85–2·8 mm.; maximum width 45–60μ.

Oesophagus : length 0·8–1 mm.

Spicule, bursa or any other copulatory organ absent. Cloaca terminal.

Host : *Epimys norvegicus.*

Habitat : Nasal cavities.

Origin : Rome (city).

Type specimens : Deposited in the collection of the Istituto di Parassitologia dell'Università di Roma.

Differential Diagnosis

The principal morphological and biological differential characters between *Trichosomoides nasalis* and *Trichosomoides crassicauda* are the following :

1. *T. nasalis* is generally longer (16–28 mm. *T. nasalis* ; 13–17 mm. *T. crassicauda*).

2. Oesophagus is longer in *T. nasalis* (2·8–3·35 mm. in *T. nasalis* ; 1·9–2·3 mm. in *T. crassicauda*).

3. Serrations are present in the cuticle of the prevulvar region of *T. nasalis*, but they are not so big as in *T. crassicauda.*

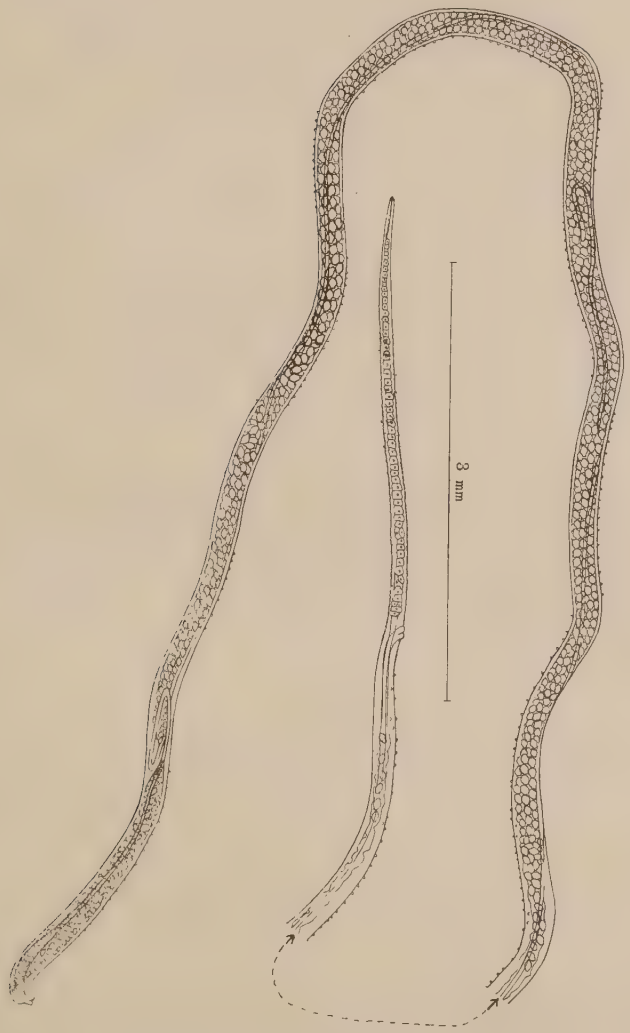


Fig. 1.—*Trichosomoides nasalis* n.sp.

4. Papilla-like cuticular elevations are present in the ventral region following the vulva to near the posterior end of the body in *T. nasalis* ; only for a distance of few mm. in *T. crassicauda.*

5. Posterior end of the body is generally bent ventrally in *T. nasalis* ; posterior end is not ventrally bent in *T. crassicauda.*

6. Eggs are generally more elongated in *T. nasalis* than in *T. crassicauda* (0·038–0·048 × 0·065–0·084mm. in *T. nasalis* ; 0·042–0·050 × 0·064–0·072mm. in *T. crassicauda*).

7. *T. nasalis* is a parasite of the first respiratory tract, *T. crassicauda* of the urinary tract of rodents.

A character considered till now of systematic importance for the definition of the family Trichosomoididae Yorke and Maplestone, 1926, is the *habitat* in the urinary tract of rodents. With the description of this new species, parasitic in the first respiratory tract, this character has lost its general systematic importance. For this reason we propose to modify slightly the definition of the family in the following manner.

Trichosomoididae Yorke and Maplestone, 1926

Definition : Male much smaller than the female and found in the vagina or uterus of the latter ; spicule and copulatory sheath absent. Female : vulva near posterior end of oesophagus. Oviparous : eggs lemon-shaped, containing embryos when deposited. Parasites of urinatory or *first respiratory tract of* rodents.

Summary

The authors describe *Trichosomoides nasalis* n.sp., parasitic in the nasal cavities of *Epimys norvegicus* and propose to modify slightly the definition of the family Trichosomoididae Yorke and Maplestone, 1926, especially in relation to the definition of the *habitat* (considered till now to be exclusively in the urinary tract).

Acknowledgment

We are indebted to Miss T. Crapulli for collecting the parasites.

Reference

Aurizi, A., 1958.—"Su un nematode del genere *Trichosomoides* parassita delle cavità nasali dei ratti di fogna (*Epimys norvegicus*) di Roma." *Nuovi Ann. Ig. Micr.*, **9**, 264–266.

Yorke, W. and Maplestone, P. A., 1926.—The Nematode Parasites of Vertebrates.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 9–16.

The Laboratory Diagnosis of Bilharziasis

W. K. Blackie, M.D., Ph.D., F.R.C.P. (Edin.)

Physician, Salisbury, S. Rhodesia

Bilharziasis is unquestionably one of the major parasitic diseases of Southern Africa where both the urinary and intestinal forms occur. When it presents in the classical form, the diagnosis either from the clinical or laboratory viewpoint is seldom a major problem ; but when, as so often happens, the presenting features have little in common with the accepted text-book descriptions of the disease, diagnosis becomes a matter of considerable difficulty.

The intermediate snail host is widely distributed throughout Southern Africa hence it is now generally agreed that the majority of the rivers and dams are infected even though genuinely "safe" areas do exist. In spite of the widely dispersed snail population massive infections are exceptional especially amongst Europeans so that the diagnostic problems centre round the relatively light infections in which local signs and symptoms are often transitory or entirely subclinical, hence the whole pattern of the disease is essentially an expression of toxaemia rather than local trauma. A brief appreciation therefore of the clinical features of bilharziasis as met with in Southern Africa is entirely relevant to the problem of laboratory diagnosis.

Penetration of the skin by bilharzial cercariae usually passes unnoticed or at most the patient is aware of a local irritation of varying intensity with sometimes a transitory point of erythema to mark the point of entry.

In a few instances invasion is associated with the Katayama syndrome in which pyrexia, urticaria, abdominal pains and general constitutional upset make up the presenting clinical picture and it is in such cases that laboratory investigation proves most rewarding.

But as already stated the invasive stage generally passes undetected and nothing happens for some 10 to 12 weeks when the presence of blood in the urine or the passage of blood-stained mucus in the stool provide clinical evidence which lends itself readily to accurate laboratory diagnosis.

On the other hand it frequently happens that in the clinical investigation of a suspected (and subsequently proven) case of bilharziasis no evidence can be elicited to suggest irritation either of the bowel or urinary bladder. In such cases the entire clinical picture constitutes an expression of toxaemia with nothing to suggest tissue damage at the definitive sites. Instead there emerges a clinical triad which, though familiar to all who have studied bilharziasis in Southern Africa, is seldom accorded a place in the systematic descriptions of the disease.

The presenting feature is invariably lassitude in which the patient complains of persistent tiredness both physical and mental. Furthermore the lassitude is completely refractory to all forms of "tonic" therapy.

Secondly, he suffers from a vague dyspepsia with or without upper abdominal pain, which again proves resistant to all forms of symptomatic treatment. In such circumstances he has usually been regarded as suffering from peptic ulceration or chronic cholecystitis in spite of an absence of radiological support.

Finally, considerable disability is occasioned by a low backache which again persists in spite of adequate physiotherapy and as a rule continues long after the completion of a course of anti-bilharzial treatment.

Such then are the clinical types of bilharziasis which a diagnostic laboratory may be called upon to investigate and it is now proposed to discuss methods available and how they can be applied to best advantage. It is not proposed to deal here with the late and complicated signs of the disease associated with gross tissue fibrosis, neoplastic change or secondary bacterial infection as in these circumstances a critical assessment necessitates both radiological and elaborate manipulative procedures.

The Routine Laboratory Investigation of Bilharziasis

The routine laboratory investigation of bilharziasis includes the examination of (a) urine, (b) stool, (c) blood and (d) biopsy material.

Examination of the Urine.

Where blood is being passed in the urine a specimen collected after partially emptying the bladder or after a brief spell of vigorous exercise will usually show bilharzia eggs. Associated findings will include the presence of a small amount of albumin and a variable number of red blood cells.

In cases of exposure to bilharzial infection in whom, however, there is no history of haematuria, dysuria or any specific bladder complaint a much more reliable procedure consists in collecting all specimens of urine passed over a period of 24 hours. The entire volume is then subjected to an ingenious filtration technique elaborated by Dr. G. V. Blaine as follows :

A simple filter is prepared by cutting away the base of an empty fruit-juice bottle of about 300 cc. capacity. A perforated cap attachment is linked with the ordinary suction apparatus of the laboratory tap and a piece of (XXX) bolting silk is inserted into the perforated cap which is screwed into the prepared bottle now fixed in a stand with the open base upwards. The 24-hour sample is then poured into the bottle, suction is started, and when filtration is complete (the whole process taking only a few minutes) the cap is unscrewed, the silk diaphragm removed and examined under the low power of the microscope. After a little practice bilharzia eggs can readily be detected.

This is undoubtedly a most valuable method of detecting eggs in lightly infected individuals, but no matter what method of examination is employed specimens must be repeated at least half a dozen times on several occasions before it is reasonable to assume freedom from infection.

Examination of the Stool

It is but seldom that blood and mucus appear in the stool in intestinal bilharziasis but when this does occur and when blood and mucus are contained in the specimen submitted for examination bilharzia eggs will usually be found without great difficulty. The usual experience however is that the stools present no macroscopic abnormalities whatsoever. In these circumstances samples should be taken from the surface of the stool, at 3 or 4 different places. The resultant specimen is emulsified in normal saline and passed through a course strainer. The filtrate is then centrifuged, the deposit is resuspended in a little saline and examined for eggs.

An alternative method of examination consists in straining the stool specimen as already described. Thereafter the filtrate is washed several times in normal saline until the supernatant fluid is clear. After sedimentation the saline is rapidly replaced by one or two changes of water, and the specimen is then examined with a hand lens against a bright light when miracidia can be detected with little difficulty.

A single negative finding by either procedure of course does not exclude bilharziasis.

Examination of the Blood

The blood count

In spite of the fact that no consistent blood picture can be ascribed to bilharziasis the white cell and differential counts frequently provide indirect evidence of the disease by reason of the increase in the eosinophile percentage.

The behaviour of the eosinophiles in the presence of bilharzial infection is by no means constant. As a general rule both in man and in experimental animals an eosinophilia develops within 6 to 8 weeks of infection and during the stage of tissue invasion the total white cell count may increase to between 15,000 and 25,000 cells per cu. mm. with the eosinophiles present in a concentration of between 20 and 60%.

These arresting figures however are not maintained indefinitely but gradually revert to near normal over the succeeding months so that by the end of a year or 18 months the white cell count may have few if any distinguishing features.

On the other hand it is true to say that in a proportion of cases a low grade eosinophilia persists and when other causes of eosinophilia have been excluded, may constitute indirect evidence of the disease. Reduction in the haemoglobin value of the blood is not unusual in longstanding cases of bilharziasis but is probably a reflection of toxaemia and its effect on haemopoiesis rather than a sequel to blood loss. It obviously has no diagnostic value and is only of importance from the point of view of adequate treatment. The conclusion to be drawn from a study of the blood in bilharziasis is that a high white cell count with an absolute eosinophilia is commonly associated with the invasive stage, whereas in the established disease a white cell count within the normal range but with a low grade increase in the eosinophile percentage may constitute indirect evidence.

On the other hand the absence of eosinophilia does not exclude a diagnosis of bilharziasis.

The Complement Fixation Test

In some centres the bilharzia complement fixation test constitutes a routine procedure in the diagnosis of bilharziasis. It was first shown by Fairley (1919) that in the presence of bilharzial infection circulating antibody appears within 3 weeks of infection. Thereafter the

titre continues to rise for several weeks to be followed by a steady fall, but so long as infection persists specific antibody can be demonstrated in at least 85% of cases. In the detection of antibody the complement fixation test is employed.

In the original tests Fairley employed an antigen prepared from infected snail livers, and in spite of its many impurities he was able to claim that "in early diagnosis that is, prior to the onset of localizing lesions in the bladder and rectum, this is the only real method of diagnosis".

More recently antigens prepared from adult worms, miracidia and cercariae have been in current use in an attempt to enhance the sensitivity of the first method.

Using cercarial antigen Lurie and de Meillon (1952) confirmed Fairley's work by demonstrating complement-fixing antibodies in monkeys (*Cercopithecus aethiops pygerythrus*) experimentally infected with *S. bovis* and *S. mansoni*. Positive results were obtained from $1\frac{1}{2}$ to $6\frac{1}{2}$ weeks from the time of infection and remained positive throughout the 9 months period of study.

In 3 human cases studied at the same time and in whom the date of infection was accurately known the complement fixation test became positive within 3 weeks of exposure to infection.

In the present state of our knowledge it may be said that the complement fixation test becomes positive during the invasion stage and has the advantage over the eosinophile response that not only is it a more reliable response but occurs about three weeks earlier. Once the infection is fully established with the parasites at their definitive sites and with oviposition in progress the reliability of the complement fixation test is more open to question. There can be little doubt that fluctuation in antibody titre occurs so that even in the presence of active infection the test may vary between positive and negative in the same individual. The same phenomenom may be observed in evaluating the question of cure following a course of anti-bilharzial therapy. This was clearly demonstrated by de Meillon and Hollingham (1958) in a small group of school children after an effective course of treatment. The drift towards consistently negative reactions was essentially erratic both in the group as a whole and in the individual members. With these recent observations in mind it is obvious that the complement fixation test cannot be regarded as infallibly positive in the established disease. Thus a single negative result is of negligible diagnostic value.

In the same way when used as a test of cure a consistently negative result up to at least 9 months after the cessation of treatment is probably a minimum requirement.

With these limitations in mind the complement fixation test is still of great diagnostic value in the investigation of bilharziasis.

Examination of Biopsy Material

Where adequate hospital and laboratory facilities exist the collection and examination of biopsy material becomes a practicable procedure in the investigation of bilharziasis. Biopsy tests are especially valuable in cases consistent with chronic bilharziasis in whom, however, the routine examination of stool and urine specimens has been uniformly negative.

The tissue for examination is obtained by direct vision either from the rectum or urinary bladder or if necessary from both sources.

Rectal Biopsy

A rectal biopsy is carried out with the aid of either a sigmoidoscope or a proctoscope but preferably the former. With judgment and experience, specimens of good diagnostic value can be obtained by removing a portion of mucosa and associated submucosa from any portion of the rectum that looks at all abnormal such as a localized patch of congestion or infiltration. Even gentle curettage or biopsy of apparently normal looking mucosa may provide valuable diagnostic information. Material obtained in this fashion is then examined in one of two ways. The tissue may be teased out in normal saline, pressed between two glass slides, and examined under the low power of the microscope, when eggs, provided they have not been grossly fragmented, can usually be identified. Alternatively the tissue may be treated as a histological specimen and serial sections prepared at 200μ intervals. This latter method has the advantage that the bilharzial lesions can be identified with accuracy even though species identification may seldom be possible. It has the added advantage that where the suspected area is found not to be bilharzial in origin its true character can be identified histologically.

Rectal biopsy certainly provides a rapid method of establishing a diagnosis in bilharziasis and although it is sometimes regarded as a useful test of cure its value in this regard is more debatable.

Urinary Bladder Biopsy

Tissue from the urinary bladder can readily be obtained in the course of a cystoscopic examination and in view of the histological response of the bladder to bilharzial infection the selection of appropriate material for laboratory investigation can be made with great precision. While the yellowish submucous nodules or the porcelain-white papule or the sandy patch invariably yield positive evidence of the disease, it is also worth remembering that a sample of apparently normal mucosa may contain eggs. Furthermore eggs occasionally appear in urine passed immediately after instrumentation of the bladder and ureters. Tissue obtained in this way is usually teased out in saline and examined between glass slides.

Where further information concerning the response of the bladder wall to the egg deposits is required the material would then be examined histologically.

Commentary

The laboratory diagnosis of bilharziasis may be an extremely simple procedure or may necessitate an elaborate and painstaking investigation, depending in large measure on the phase of the disease and the intensity of the infection. During the stage of invasion which is sometimes associated with what the clinicians describe as the Katayama syndrome, the parasite is immature and oviposition at the definitive sites has not yet begun hence the examination of urine and stool specimens has no diagnostic value. But as it has been shown that a vigorous and sustained antibody response develops at this time, the complement fixation test constitutes the first and most reliable evidence of bilharzial infection.

A white cell count would also show significant changes at this time in that there is usually a well marked eosinophilia in association with a raised total count, but when taken by itself and unsupported by the serological test this finding can only be regarded as presumptive evidence of the disease.

During the clinically overt phase of the disease when the parasite has reached its definitive site and the mucosa of the bowel or urinary bladder is being traumatized by the erupting eggs, the presence of haematuria or mild dysenteric symptoms indicate a simple and straightforward approach to the diagnosis. In these circumstances the careful selection of stool and urine specimens will readily provide

positive evidence of the disease even though the examination may have to be extended beyond a single specimen. When the infection has entered a more chronic phase in which toxaemic rather than traumatic or irritative features predominate a more elaborate investigational programme has to be followed.

The examination of stool and urine specimens is still an integral part of the programme provided some concentration technique is employed. In the case of the urine this consists in examining a 24 hour collection by the filtration technique, while stool specimens are sieved and centrifuged.

When negative results are obtained a rectal biopsy is indicated. This is an undertaking which presents no special difficulties either in the doctor's consulting room or in hospital and only very rarely is severe haemorrhage a complication. It is applicable to all forms of the disease.

Using a sigmoidoscope 3 specimens are taken, one from each of the valves of Houston. According to Prata (1957) 15% at least of positive cases are missed if one snip only is taken. The material is teased out in normal saline and examined between two glass slides. It is probable that about 60% of cases can be diagnosed in this way but again a negative result does not exclude the disease.

Finally, with the specimen tests and the rectal biopsy negative, the bilharzia complement fixation test, repeated if necessary, may at last provide evidence of the disease.

References

Fairley, N. H., 1919.—"The discovery of a specific complement fixation test for bilharziasis and its practical application to clinical medicine." *J. R. Army med. Cps.*, **32**, 449–460. (W.L. 11035).

Lurie, H. I., de Meillon, B. and Stoffberg, N., 1952.—"Experimental bilharziasis in animals. I. Early diagnosis of bilharziasis." *S. Afr. med. J.*, **26**, 1005–1008. (W.L. 20359).

de Meillon, B. and Hollingham, E. A., 1958.—"Observations on the Bilharzia complement-fixation test in schoolchildren after successful treatment." *Bull. World Hlth. Org.*, **18**, 1108–1109. (W.L. 5748c).

Prata, A., 1957.—Biópsia retal na esquistossomose mansoni. Bases e aplicaçoes no diagnóstico e tratamento. Serviço Nacional de Educaçao Sanitária, Rio de Janeiro.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 17–24.

Anomalous Results from an Experimental Infection of Man with *Brugia malayi* (Brug, 1927)

By J. J. C. Buckley and R. H. Wharton

From the London School of Hygiene and Tropical Medicine and the Institute for Medical Research, Kuala Lumpur, Federation of Malaya

Previous attempts (Buckley, 1958) to infect a volunteer with *Brugia malayi** from a monkey (*Macaca irus*) and *B. pahangi* from a domestic cat, failed in both cases to produce a mature infection, but resulted in the occurrence of all** the characteristic signs and symptoms of tropical pulmonary eosinophilia. This supported the theory earlier put forward by Danaraj (1956) and Danaraj *et al.* (1957) that the disease may be caused by exposure to infection with filarial parasites of animals, for which man is an unsuitable host and in whom full development cannot take place. The theory was based on observations of numerous cases in Singapore, all of whom gave a strong reaction to the filarial complement fixation test and were cured by treatment with Banocide (diethylcarbamazine). They noted too that patients infected with *W. bancrofti* and *B. malayi* had only a moderate eosinophilia by comparison, that the F.C.F. test was positive in relatively low titres and in some only of the sera from these cases.

The experiment described here was intended to compare the effect of an infection with a filarial parasite of man with the effects previously observed of infection with filarial parasites of animal origin, using the same volunteer, (Buckley, 1958). The observations parallel those of Edeson *et al.* (1960) which will be referred to in the discussion.

* Originally published as (?) *malayi*, it has since been found to be morphologically indistinguishable from *B. malayi* from man ; it is possible however that it differs biologically from the latter form.

** In the published report of these cases the lung x-rays were stated to be normal. Subsequent re-examination of them revealed the presence of the mottling characteristic of T.P.E.

Material and Methods

Twenty-six *Mansonia uniformis* were given an infective blood meal on a cat (No. 59) which had been experimentally infected with *B. malayi* from man in Kuantan, Malaya. They were flown to London in September, 1958. Three were dead on arrival ; the remainder were kept alive for varying periods and from them a total of 70 infective larvae were recovered.† Of these 44 were injected subsequently into the volunteer. The sites of inoculation and numbers were: 21 into the left thigh, 8 into the left calf and 15 into the right calf. Five injections were given : 5 larvae on 25th September, 14 on the 27th, 2 on the 29th, 8 and 15 on the 30th. A cat as control was given 26 larvae, 10 in the right groin on the 29th September and 16 in the left groin on the 30th.

Results

About the time of the inoculation (2nd October) the percentage of eosinophils was about 2 per cent. and weekly counts were done subsequently during the course of the experiment. In the 4th week the percentage rose above normal (13%) and thereafter gradually rose, but with several fluctuations, to a peak of 74% on the 1st April, 1959, a period of about 26 weeks. Treatment with Banocide was begun on 1st April, after which the eosinophil curve performed the usual headlong dive to near normal but remained between 8 and 17 per cent. during the next 8 weeks. Total white blood cell counts on 4 occasions showed that the blood picture change was in fact an eosinophilic leucocytosis. (See Graph I).

Filarial complement fixation tests were done by Dr. D. S. Ridley at the Hospital for Tropical Diseases on five occasions (see graph) and positive reactions occurred four times, the highest titre being 1:160. The fifth test, in July 1959, gave a weak reaction within normal limits (1·5±). A skin test with filarial antigen on 14th October 1958, was strongly positive and caused considerable swelling of the whole of the left arm.

† Success in transporting infected mosquitos from Malaya to London came at the fifth attempt. Earlier efforts failed for various reasons—at first mosquitos were kept in Kuantan for 7–8 days after feeding so that they would arrive in London with infective larvae, but though *Armigeres obturbans* infected with *B. pahangi* were previously sent this way almost all the *Mansonia* died on the way. Later it was found that mosquitos which had recently fed survived the journey satisfactorily. They were placed individually in polythene tubes, 1 in.× ½ in., with a close fitting cap containing a small wad of moistened toilet paper in the top, then packed inside a small tin lined with cotton wool and forwarded by air-freight.

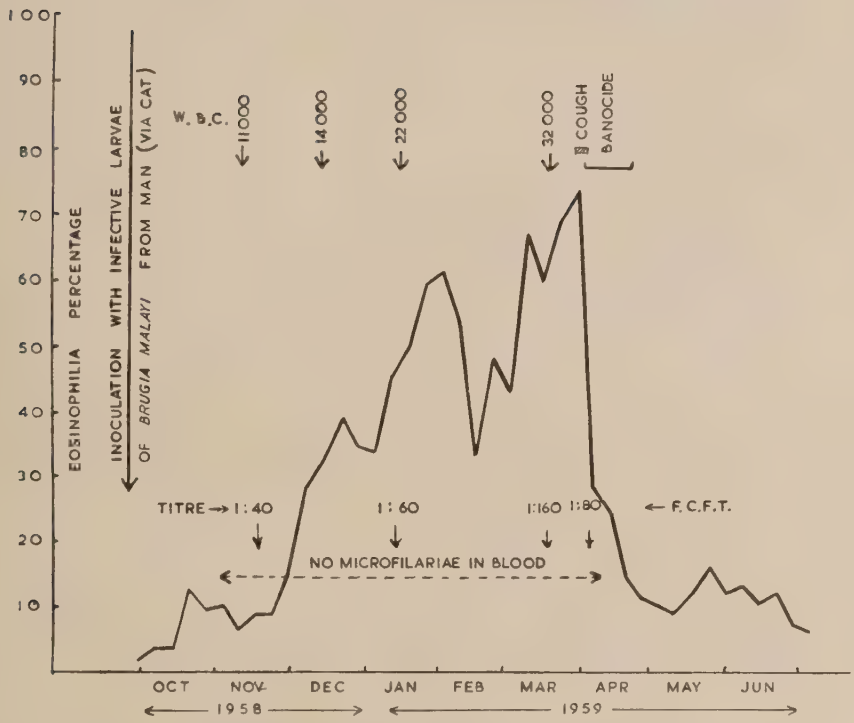


Fig. 1.—Showing percent eosinophils and other data following an experimental infection of a volunteer with *B. malayi*.

A chest X-ray done on 1st April 1959, just prior to Banocide treatment, showed no recent radiological changes and appearances were unchanged in another one done on 28th May. We are greatly indebted to Professor A. W. Woodruff, and other members of the staff of the Medical Unit, Hospital for Tropical Diseases, for the treatment of this case and the clinical details.

Weekly examinations of thick blood smears (60 cu.mm.) from 3rd November 1958 up to the time of treatment with Banocide were negative for microfilariae. The cat control also remained negative during this period and on subsequent occasional examinations of its blood.

Clinical changes occurred during the experiment. On 5th November (5 weeks after inoculation) a lump was noticed in the left popliteal region ; it disappeared after a few days. On the 6th December (9 weeks) there was a transitory oedematous swelling of the left ankle and on the 19th January (14 weeks) there was a transitory swelling on the right thigh, about 6 inches above the knee. Of particular interest was the rather sudden development of a distressing wheezy cough which was identical with that which followed the previous inoculations with filarial infections from a monkey and a cat. It coincided with the ‡observed peak development of the eosinophilia, circa 70%, about 25 weeks (Fig. 2C) after the original inoculation. (c/f. 14 and 10 weeks respectively in the previous experiment). (See Fig. 2A and B).

Discussion

The "anomalous results" from this experiment relate mainly to the theory that T.P.E. is caused by exposure to infection with filarial parasites of animal origin. In this respect the unexpected results were (a) the occurrence of the typical T.P.E. cough, (b) the high eosinophilia with leucocytosis, (c) the strong reaction to the complement fixation test, (d) the non-appearance of microfilariae in the blood.

(a) This was unexpected and did not conform with Danaraj's evidence that normal (non-animal) filarial infections in man do not give rise to this aspect of T.P.E. It might be argued however that since the volunteer had previously been exposed to filarial infections (of animal origin) he had become and remained sensitized, although there had been complete return to normality in the blood and serum picture. It suggests the possibility that persons who may become sensitized by filarial infection, of either human or animal origin, are likely subjects for T.P.E. on a subsequent infection. This however is purely speculative and needs further investigation.

‡ The true peak is unknown as Banocide treatment was begun at this time.

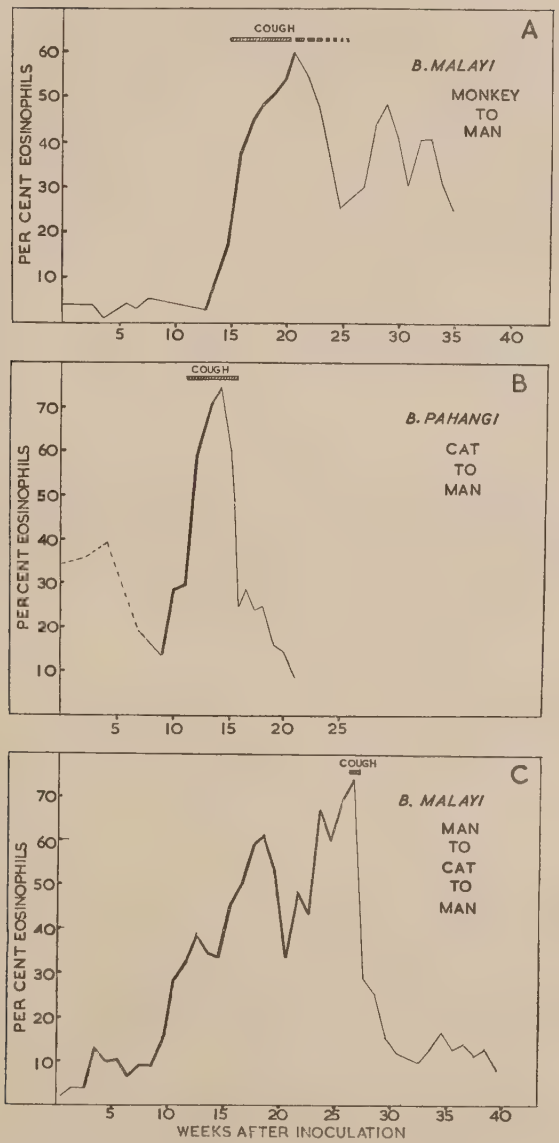


Fig. 2.—Three graphs showing the course of the eosinophil curve following experimental infection of a volunteer with *Brugia* spp. from animals (A and B) and with *B. malayi* from man (C).

(b) The high eosinophilia and leucocytosis, which are not usually associated with *B. malayi* infections in man (c/f. Danaraj) could also be explained on the grounds of pre-sensitization. It is of interest to note the course of the eosinophil curve in this case. It began to rise early (2–3 weeks) after the inoculations : its rise was gradual, discontinuous and took a long time, 26 weeks, to attain 74%. In the previous experiment the eosinophil curve did not begin to rise until, respectively, 14 and 10 weeks after the inoculations and then the rise was very rapid and was continuous, taking only about 6 and 5 weeks respectively to attain the observed maximum.

(c) The strong reaction to the complement fixation test which as Danaraj pointed out is not a feature of *B. malayi* infection in man is subject to the same comments as (a).

(d) The non-appearance of microfilariae in the blood in this case is the least anomalous of the results and tallies with the well-known but unexplained fact that in a community having a high microfilaraemia rate and a high exposure risk to the bites of infected mosquitos there are always some persons whose blood does not contain microfilariae*. The non-appearance of the microfilariae in the control cat does not call for special comment in view of the occasional negative results obtained on previous occasions.

The "zoonotic" theory of the aetiology of T.P.E. has not received support from the experiments on volunteers by Edeson *et al.* (1960). Neither of the two volunteers inoculated with the "animal" species, *B. pahangi*, showed signs or symptoms of this disease. In fact since one of the volunteers (with *pahangi*) developed microfilariae, the behaviour of the parasite and the host reaction to it in this case was close to what one would expect of a *malayi* infection of human origin. Commenting on their results these authors wrote : "Parasitism involves a host as well as a parasite, and the end result of any infection may be determined more by the host's response than by the strain of parasite." On the other hand, it may not, and the strain of parasite *may* be just as important or even more important than the host in determining the result of such infections. In the present state of our knowledge (or ignorance) it is surely unwise to "take sides" on the importance of these two factors. It has recently been found (Wilson *et al.*, 1958) that there are at least two "strains" of *B. malayi*

* It is remarkable too that in the experiments by Edeson *et al.* (1960) the two volunteers who had been inoculated with *B. malayi* never showed microfilariae in their blood.

in Malaya. It is not impossible, therefore, that more than one strain of *B. pahangi* also exist, e.g., one which can infect both man and animals and produce in man the signs and symptoms characteristic of a *malayi* infection as in Edeson's experiment and another strain to which man is refractory and results in the exaggerated reactions exemplified in tropical pulmonary eosinophilia, as in Buckley's experiment. Speculation on these lines might also be extended to *W. bancrofti* infections to "explain" the occurrence of occult infection of man with this species (see Webb *et al.* 1960), and its association with T.P.E. In view of all these uncertainties it is fitting to quote again from the last paragraph of the article by Edeson *et al.* : "Only when experiments like these can be repeated on a larger scale with various filariae will we know which of the varying reactions to filarial infection are of real importance to a better understanding of the naturally acquired disease."

Summary

A volunteer who had been previously exposed to infection with filarial infections from naturally infected animals, i.e., *Brugia malayi* from *Macaca irus* and *B. pahangi* from a domestic cat, was exposed to infection with *B. malayi* from man. He developed signs and symptoms of tropical pulmonary eosinophilia and transitory swellings of the lower limbs, possibly of filarial origin. Up to the time of treatment with Banocide no microfilariae were found in his blood.

We have great pleasure in contributing this article to the Birthday number in honour of Professor Leiper and in offering him our hearty greetings and good wishes.

References

Buckley, J. J. C., 1958.—"Tropical pulmonary eosinophilia in relation to filarial infections (*Wuchereria* spp.) of animals." *Trans. R. Soc. trop. Med. Hyg.*, **52**, 335–336. (W.L. 21671.)

————, 1958.—"Occult filarial infections of animal origin as a cause of tropical pulmonary eosinophilia." *E. Afr. med. J.*, **35**, 493–500. (W.L. 7522c.)

Danaraj, T. J., 1956.—"The treatment of eosinophilic lung (tropical eosinophilia) with hetrazan (a preliminary report)." *Proc. Alumni Ass. Malaya*, **9**, 172–187. (W.L. 16598a.)

————, da Silva, L. S., and Schacher, J. F., 1957.—"The filarial complement-fixation test in eosinophilic lung (tropical eosinophilia)—a preliminary report." *Ibidem*, **10**, 109–116.

EDESON, J. F. B. and WHARTON, R. H., 1958.—"The experimental transmission of *Wuchereria malayi* from man to various animals." *Trans. R. Soc. trop. Med. Hyg.*, **52**. 25–45. (W.L. 21671.)

————, WILSON, R., WHARTON, R. H. and LAING, A. B. G., 1960.—"Experimental transmission of *Brugia malayi* and *B. pahangi* to man." *Ibidem*, **54**, 229–234.

WEBB, J. K. G., JOB, C. K. and GAULT, E. W., 1960.—"Tropical Eosinophilia. Demonstration of Microfilariae in Lung, Liver and Lymph-Nodes." *Lancet*, **1**, No. 7129, 835–842. (W.L. 11996.)

WILSON, T., EDESON, J. F. B., WHARTON, R. H., REID, J. A., TURNER, L. H. and LAING, A. B. G., 1958.—"The occurrence of two forms of *Wuchereria malayi* in man." (Correspondence). *Trans. R. Soc. trop. Med. Hyg.*, **52**, 480. (W.L. 21671.)

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 25–34.

On a Phylogenetic Classification of the Family Trichostrongylidae Leiper of Marsupials, Insectivores and Edentates

By Thomas W. M. Cameron and Betty J. Myers
Institute of Parasitology, McGill University

Leiper in 1908, created the sub-family Trichostrongylinae in his new family Metastrongylidae, for those small intestinal bursate nematodes without a buccal capsule. It included the genera *Trichostrongylus*, *Haemonchus*, *Nematodirus*, *Cooperia* and *Ostertagia*. In 1912, he raised the status of this sub-family to family rank as Trichostrongylidae Leiper, 1912. All the original genera in this family have a double ovarian system in the female ; *Heligmosomum* described by Railliet and Henry in 1909, has a single ovary and in 1914, Travassos created a new sub-family Heligmosominae for this and related species.

The trichostrongyles are well represented in domestic ruminants in which they are of major economic importance and in the ensuing years, a great many different species were described ; accordingly in 1927, Cram created the super-family Trichostrongyloidea for the group and raised the Heligmosominae to family rank as Heligmosomidae Cram, 1927.

The number of species described continued to grow and at present over 350 species of the Trichostrongylidae alone have been described —many of them inadequately. As a result, the classification of the group has become extremely confused. Travassos has twice, in 1921 and 1937, monographed the family while Skrjabin, Shikobalova and Shul'ts more recently (1954) have done the same thing. The latter authors have divided it into 15 subfamilies containing 18 tribes, in an attempt to systematize the classification, which because of the general simplicity of the group, has been based on characters, often arbitrarily chosen, which could be used in rapid identification.

As a parasite is essentially an animal which has a host, in many cases the host-parasite relationship has become a very specific one. An examination of the hosts of the trichostrongyles has shown that the majority of the members of this family can be grouped according to the relationship of their hosts although admittedly, this relationship is not complete ; moreover, it can be assumed that many more members of this family have still to be described. Some genera have

a wide host range but this is exceptional. However, it is probable that such genera have a somewhat generalized physiology and non-specific or slightly specific host requirements. We believe, however, that the information in the three major publications mentioned above can be rearranged on a more phylogenetic basis. We have accordingly examined the members of the Trichostrongylidae from the point of view of their host's phylogeny. We have disregarded experimental laboratory-type infections, as well as *nomina nuda*. In some cases, descriptions are inadequate and the classification of such species must remain tentative pending a re-examination of material from the same hosts.

We have restricted this review to the members of the family Trichostrongylidae which occur in the archaic South American host groups and some possibly related hosts. South America was isolated during most of the Tertiary from the rest of the world when it developed its own peculiar fauna of edentates and opossums. Both orders flourished during most of the Tertiary and gave rise to a great many species but both are now reduced to very small proportions. The edentates include the sloths, the anteaters and the armadillos while the marsupials include the opossums and a few close relations. The parasites of the platyrrhine monkeys and the hystricomorph rodents were probably not members of the original South American mammalian fauna but arrived about mid-Tertiary times from Africa and, therefore, do not fall within the purview of this article. However, we have also considered the parasites of the archaic West Indian insectivore, *Solenodon* and, because of its possible affinities with the Edentata, those of the Africo-asiatic pangolins which may have evolved from the Proto-Edentata.

Because of their small size and simple morphology, morphological distinctions between genera in the Trichostrongylidae are not only limited but are often slight. A careful examination of possible details which can be used to trace relationships, suggests that they should be limited, in generic characters at least, to : the shape of the head and the presence or absence of longitudinal lines on the body, the position of the female genital opening, the course taken by the ovaries, and the shape of the female tail ; the shape and symmetry of the male bursa, the presence or absence of an accessory bursa, the distribution of the bursal rays, and the relative shape and size of the spicules.

The "rays" in the bursa are elongated structures carrying nerves from the lateral trunk. The dorsal ray is a fused structure consisting of *two* lateral dorsal rays, more or less split through part or all of their length. Its terminal portion—which carries a single nerve on each

side—is often split into two or three portions and small variations are frequent ; it is apparently still a quite labile structure and presumably, the more primitive condition is that in which there are *two* dorsal rays, which would imply either two dorsal lobes or none at all.

On the lateral aspect of each dorsal ray, is an externo-dorsal ray, so-called because its nerve ending is on the exterior surface of the bursa. This ray is always in the lateral lobes somewhere in the vicinity of the lateral group of rays.

The latter group consists of three rays, the postero-lateral, the medio-lateral, and the externo-lateral ; the latter has its nerve ending on the exterior surface of the bursa. The postero- and medio-lateral rays are usually more or less close together, but their tips may diverge and the medio-lateral ray may be straight or turned towards the ventral rays. The externo-lateral ray may or may not be close to the others but its termination is often some distance from the margin of the bursa. The three rays may be thin or stout or may have different thicknesses.

The ventral group consists of two rays which may be of different thickness and may be parallel to each other or diverge wholly or in part. The ventro-ventral ray (that is, the most anterior) is directed anteriorly, but the latero-ventral ray may be straight, directed dorsally and may be close to the lateral group.

Prebursal papillae are probably always present although when small they are often not observed. They may be ray-like.

The accessory male genital organs are spicules, gubernaculum, and telamon. The spicules may be simple or complicated, short or long, similar or dissimilar, but often vary from species to species within a genus. A spicular membrane is probably always present, but being transparent is often not observed unless the spicules are dissected out. A gubernaculum is simply a terminal portion of the male canal, cuticularized to act as a guide for the extrusion of the spicules. If not pigmented it is not often observed and there is reason to doubt statements that it is absent. The telamon is the cuticularized dorsal edge of the canal and acts as a shield so that sharp pointed spicules can be protruded easily. It, like the gubernaculum, is probably always present although often not pigmented.

The male accessory organs are of less value in determining phylogenetic relationships than the bursa, and the gubernaculum and telamon are probably least important of all, because when structures are small or unpigmented they are not seen and the so-called absences of these often mean only that they were not

observed, as in the reported presence or absence of papillae ; many descriptions are quite unreliable on these points and it is accordingly often misleading to consider them in a phylogenetic classification. On the basis of the features we considered reliable, we have classified these nematodes into several tribes. We have included all described species, although it is doubtful if all are really valid.

The South American trichostrongylids have been placed in many different subfamilies by Skrjabin, Shikhobalova, and Shul'ts (1954) but it appears to us that these trichostrongylids (including the two genera recorded from West Indian insectivores and Old World pangolins, respectively) constitute a more compact natural evolutionary group with possibly several original primitive origins. We have regrouped them into seven tribes as follows :—

(1) Macieliea trib.nov.

Trichostrongylidae : The head in both sexes is inflated. In the female, the vulva is situated in the posterior half of the body. In the male, the bursa is trilobed and symmetrical. The dorsal ray is bifurcated in its distal half, each bifurcation having two or three digitations. The externo-dorsal ray is long and arises near the base of the dorsal ray. The medio- and postero-lateral rays lie close together, their tips bending dorsally and reaching the edge of the bursal membrane ; the externo-lateral ray is separate from them and terminates a short distance from the edge of the bursa. The ventral rays are directed anteriorly and reach the edge of the bursa. The spicules are short, complex, and multipointed and probably always have spicular membranes. A gubernaculum is present.

Parasites of Edentata and pangolins.

Type genus : *Macielia* Travassos, 1935

Other genera : *Fontesia* Travassos, 1928
Manistrongylus Cameron and Myers, 1960.

Macielia Travassos, 1935
synonym *Delicata* Travassos, 1935

Macieliea : The head is without lateral alae and longitudinal ridges are absent. The tail of the female is pointed or terminates in a single spine. In the bursa, the dorsal lobe is small, an accessory bursal membrane is present, and the ventral rays diverge distally.

Parasites of armadillos and anteaters.

Type species : *Macielia macieli* (Travassos, 1915),
synonym *Cooperia macieli* Travassos, 1915

The differences between this genus and *Delicata* seem to us to be insufficient to separate them and to be of specific value only. We have, accordingly, synonymized them, giving priority to *Macielia* because of pagination in Travassos' original publication (1935). The species in this enlarged genus, which are as follows, are separated essentially on the structure of the spicules.

Macielia appendiculata (Travassos, 1928)
synonym *Delicata appendiculata* Travassos, 1928.
M. cameroni (Travassos, 1935) n.comb.
synonym *Delicata cameroni* Travassos, 1935.
M. chagasi Travassos, 1935.
M. delicata (Travassos, 1921).
synonym *Delicata delicata* (Travassos, 1921).
M. falsa (Travassos, 1921).
M. flagellata Travassos, 1937.
M. khalili (Travassos, 1928) n.comb.
synonym *Delicata khalili* (Travassos, 1928).
M. pseudoappendiculata (Cameron, 1939) n.comb.
synonym *Delicata pseudoappendiculata* Cameron, 1939.
M. ransomi (Travassos, 1921) n.comb.
synonym *Delicata ransomi* (Travassos, 1921).
M. similis (Travassos, 1935) n.comb.
synonym *Delicata similis* Travassos, 1935.
M. uncinata (Travassos, 1935) n.comb.
synonym *Delicata uncinata* Travassos, 1935.
M. variabilis (Travassos, 1935) n.comb.
synonym *Delicata variabilis* Travassos, 1935.

Fontesia Travassos, 1928

Macieliea : The head is provided with lateral alae and longitudinal ridges are present. The tail of the female is truncated and multispined. There is no accessory bursal membrane in the male ; the ventral rays diverge distally.

Parasites of anteaters.

Type species : *Fontesia fontesi* Travassos, 1928.

The only other species in this genus is *F. secunda* Lent and Freitas, 1938.

Manistrongylus Cameron and Myers, 1960

Macieliea : The head is without lateral alae. Longitudinal ridges are absent. The tail of the female is round with a single terminal

spine. In the male bursa the dorsal lobe is well marked. The ventral rays are close together throughout their entire length.

Parasites of pangolins.

Type and only species : *Manistrongylus meyeri* (Travassos, 1937).

(2) Bradypostrongylea trib.nov.

Trichostrongylidae : The head in both sexes is inflated ; longitudinal ridges are absent on the body. In the female, the vulva is situated about the posterior quarter of the body. In the male, the bursa is symmetrical with an indistinct dorsal lobe. The dorsal ray is long, bifurcated in its distal half ; the bifurcations in turn are divided into two long, slender digitations. The externo-dorsal ray is long and slender and reaches the edge of the bursa. All three lateral rays arise close together but diverge in their distal halves to reach the edge of the bursa. The ventral rays are directed anteriorly, but also diverge. The spicules are short, multipointed and complex.

Parasites of Edentata.

Type genus : *Bradypostrongylus* Price, 1928.

Other genus : *Pintoia* Travassos, 1928.

Bradypostrongylus Price, 1928

Bradypostrongylea : The tail of the female is multipointed. The ovaries are spiralled round the digestive tube. In the male, prebursal papillae are prominent. The gubernaculum is double.

Parasites of sloths.

Type species : *B. panamensis* Price, 1928.

Pintoia Travassos, 1928

Bradypostrongylea : Lateral striations are present on the body. The gubernaculum is stated to be absent.

Parasites of anteaters.

Type species : *P. inflata* (Molin, 1861) Travassos, 1928.

This genus was placed in synonymy with the previous one by Travassos (1937), but in view of the pecularities of the female genital system in *Bradypostrongylus* and the fact that it has not been described for *Pintoia*, it seems desirable to retain it as a separate entity.

(3) Filicapitea Skrjabin and Shikhobalova, 1952

Trichostrongylidae : The anterior end is long and filiform. The oesophagus confined to anterior portion. In the female, the vulva is

near the posterior sixth of the body. In the male, the bursa is trilobed. The dorsal ray terminates in four digitations. The externo-dorsal rays are long and slender but do not reach the margin of the bursa. The lateral rays are also slender, close together, and reach the edge of the bursa. The ventral rays are massive, close together and directed anteriorly. The spicules are short, complex, and multipointed. A gubernaculum is present.

Parasites of Edentata.

Type and only genus : **Filicapitis** Travassos, 1949.

Type and only species : *F. longicollis* Travassos, 1949.

Parasites of anteaters.

(4) Trichohelicea Skrjabin and Shikhobalova, 1952

Trichostrongylidae : The head end is inflated in both sexes. In the female, the vulva is close to the anus. In the male, the bursa is trilobed. The dorsal lobe is large and shallow. The main dorsal stem bifurcates about its midpoint, each branch terminating in three digitations. The externo-dorsal ray terminates some distance from the edge of the bursa. The lateral and ventral rays all diverge from each other. The externo-lateral ray does not reach the edge of the bursa. The ventro-ventral ray is curved anteriorly. The spicules are short, similar, relatively simple with double pointed ends. A gubernaculum is present.

Parasites of Edentata.

Type and only genus : **Trichohelix** Ortlepp, 1922.

Parasites of armadillos and anteaters.

Type and only species : *T. tuberculata* (Parona and Stossich, 1901) Ortlepp, 1922.

Somewhat puzzling, however, is the reported occurrence of this species in skunks. It was originally described as *Oesophagostomum tuberculatum* by Parona and Stossich in 1901 from an edentate, *Dasypus villosus*. It was subsequently collected by Ortlepp in 1922 from the same host and made the type of the genus *Trichohelix*. Sprehn in 1927 listed *Mephitis mephitis* as a host, with no comment or description. Stiles and Baker (1935) in their check list apparently copied this record and in 1937 Travassos listed it from *Conepatus suffocans* and *Mephitis mephitis*. In the absence of any information about the original record, we are compelled to regard this as a misidentification or an accidental infection as there is no subsequent report of its presence in Mustelids, although a great many have been examined for parasites.

(5) Graphidiopsea trib.nov.

Trichostrongylidae : The head in both sexes is swollen. The tail of the female is pointed. In the male the bursa is weakly trilobed, the dorsal ray bifurcates, each bifurcation ending in two or three digitations. The externo-dorsal ray arises at the base of the dorsal and its tips are directed inwards. The lateral and ventral rays are about the same size and the tips of the medio- and postero-lateral rays are directed dorsally and reach the edge of the bursa. The externo-lateral ray terminates short of the edge of the bursa and its tip is pointed anteriorly. The ventral rays diverge in their distal halves and point anteriorly. The spicules are long and filiform. A gubernaculum is present.

Parasites of Edentata.

Type and only genus : **Graphidiops** Lent and Freitas, 1938. (synonym *Caenostrongylus* Lent and Freitas, 1938).

Parasites of anteaters.

Type species : *G. costalimai* Lent and Freitas 1938.

Other species : *Graphidiops browni* (Price, 1928)
G. inaequalis Lent and Freitas, 1938
G. major Travassos, 1949
G. ruschii Travassos, 1949
G. splendidus (Lent and Freitas, 1938) n.comb. (synonym *Caenostrongylus splendidus* Lent and Freitas, 1938).

The genus *Caenostrongylus* was distinguished from *Graphidiops* by the shorter spicules—which are still filiform. We consider this to be of specific rather than generic importance.

(6) Travassostrongylea trib.nov.

Trichostrongylidae : The head has a cuticular inflation and longitudinal crests occur on the body. The tail of the female is pointed, the vulva is in the posterior quarter of the body and protected by cuticular expansions. The male bursa has two large lateral and a small dorsal lobe. The dorsal ray is short but all others are relatively long and divergent. The spicules are equal and short. A gubernaculum is present.

Parasitic in South American Marsupialia.

Type genus : *Travassostrongylus* Orloff, 1933.

Other genus : *Camerostrongylus* Wolfgang, 1951.

Travassostrongylus Orloff, 1933

Travassostrongylea : In the male, the bursa has an accessory membrane and there are radial striations on the lateral lobe. The dorsal ray is small and bifurcates near its base. The externo-dorsal ray is thin, long, extends to the edge of the lateral lobe, and is parallel with the postero-lateral ray. The lateral and ventral rays extend to the edge of the bursa and all diverge from each other in their distal halves. The posterio- and medio-lateral rays have their tips directed away from the dorsal rays. The externo-lateral and the latero-ventral rays are more or less straight ; the ventro-ventral ray is widely separate from the latter and is bent anteriorly. The spicules are complex and have well developed membranes.

Parasitic in opossums.

Type species : *Travassostrongylus callis* (Travassos, 1914).

Other species : *T. orloffi* Travassos, 1935.
T. tertius Travassos, 1935.

Three other species from the same host were referred to this genus by Freitas in 1937. The females were unknown. The males differed in considerable detail and their descriptions suggest that they are abnormal specimens : *Travassostrongylus quatuor* Freitas, 1937, *T. quintus* Freitas, 1937, and *T. sextus* Freitas, 1937.

The dorsal ray in these individuals is very complex and irregularly divided into several digitations. The externo-dorsal ray is more slender than the others and does not reach the bursal margin. The postero- and medio-lateral rays are curved dorsally and reach the margin, but the externo-lateral ray is straight and terminates about half way towards the margin. Both ventral rays are separate but parallel throughout their length and directed anteriorly. The spicules are equal, complex and multipointed.

Camerostrongylus Wolfgang, 1951

Travassostrongylea : The lateral lobes of the bursa are asymmetrical and there is no accessory bursal membrane. The dorsal ray is small, simple, and terminates in four digitations. The externo-dorsal ray is relatively long and it, as well as the lateral and ventral rays reach the edge of the bursa. The tips of the externo-dorsal, the postero-, and medio-lateral rays are directed dorsally, while the externo-lateral and both ventral rays are directed anteriorly ; all of these rays are separate throughout almost their entire length. The spicules are simple.

Parasitic in opossums.

Type and only species : *C. didelphis* Wolfgang, 1951.

(7) Shattuckiea trib.nov.

Trichostrongylidae : The head has a cuticular inflation and longitudinal crests occur on the body. The tail of the female is pointed and the vulva is in the posterior quarter of the body. The male bursa has two large lateral and a small dorsal lobe. All rays are relatively long and slender. The spicules are equal and stout. A gubernaculum is present.

Parasitic in the archaic insectivore *Solenodon* in the West Indies.

Type and only genus : **Shattuckius** Sandground, 1938.

Type and only species : *Shattuckius shattucki* Sandground, 1938.

References

Freitas, J. F. Teixeira de, 1937.—"Sobre algumas especies do genero *Travassostrongylus* Orloff, 1933 (Nematoda : Trichostrongylidae)." *Mem. Inst. Osw. Cruz*, **32**, 217–220. (W.L. 13465).

Leiper R. T., 1908.—"An account of some helminthes contained in Dr. Wenyon's collection from the Sudan." *3rd. Rep. Wellcome trop. Res. Lab.*, 187–199. (W.L. 18653).

————, 1912.—"Check-list of helminthes parasitic in cattle." *J. Lond. Sch. trop. Med.*, **1**, 115–123. (W.L. 11299).

Skrjabin, K. I., Shikhobalova, N. P. and Shul'ts, R. S., 1954.—"Principles of nematodology, edited by K. I. Skrjabin. Vol. III. Trichostrongyloidea of animals and man". Moscow : Izdatelstvo Akademii Nauk SSSR, 683 pp.

Sprehn, C. E. W., 1927.—"Nematoden in Säugetieren." *Z. Säugetierk.*, **2**, 38–67. (W.L. 23562a).

Stiles, C. W. and Baker, C. E., 1935.—"Key-catalogue of parasites reported from Carnivora (cats, dogs, bears, etc.) with their possible public health importance." *Nat. Inst. Hlth. Bull.*, **163**, 913–1223. (W.L. 14829).

Travassos, L. P., 1921.—"Contribuições para o conhecimento da fauna helmintolojica brasileira. XIII. Ensaio monografico da familia Trichostrongylidae Leiper, 1909." *Mem. Inst. Osw. Cruz*, **13**, 1–135. (W.L. 13465).

————, 1935.—"Alguns novos generos e especies de Trichostrongylidae." *Rev. med.-cirurg. Brazil*, **43**, 345–361. (W.L. 18910).

————, 1937.—"Revisão da familia Trichostrongylidae Leiper, 1912." *Monog. Inst. Osw. Cruz*, Vol. I and II. 512 pp.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 35–40.

Recent Observations on Helminthiasis in some British Game Birds

By Phyllis A. Clapham, D.Sc.*
Wellcome Veterinary Research Station, Frant, Sussex

Between 1947 and 1958, many birds were sent in to the Game Research Station of Imperial Chemical Industries Ltd. at Fordingbridge, for post-mortem examination and diagnosis of the cause of death. They were sent from almost every county of England, though more came from Hampshire and from the eastern counties than from elsewhere. Scotland provided a few, mostly from the Lowlands but Wales is, unfortunately, almost unrepresented. All the birds reported upon here had been found either dead or dying, and none that had been shot in the ordinary way are included.

As a result of the wide geographical distribution of the post-mortem cases, it can fairly be said that these birds represent as typical a sample of British game birds as we are likely to get. I am indebted to all the land owners, estate agents and keepers who made this material available to me.

The entire intestinal tract, from mouth to anus, was examined but was separated into the following regions—mouth, oesophagus and crop, proventriculus, gizzard, duodenum and jejunum, small intestine, caeca and large intestine. The trachea and lungs were examined separately. It was not possible to examine the blood as this was always congealed, and had undergone much decomposition before the birds reached me.

The helminths of partridges and pheasants are individually considered below.

Grey partridge (*Perdix perdix*)

A total of 2,694 grey partridges was examined in this period, including 532 chicks under 2 weeks old, which have little helminthological interest, leaving 2,162 for further consideration. During the first 5 years of the work of the Station, most of the birds were wild ones but since then, partridge rearing has been put on a commercial basis so more penned birds came in for examination and the pattern of disease changed slightly as a result.

* Formerly of the Game Research Station, Imperial Chemical Industries Ltd., Fordingbridge, Hampshire.

Helminth parasites were found in 964 of the birds over 2 weeks old, i.e. in 44·6 per cent. Many of these were multiple infestations which at times included 4 different species. There were 402 deaths which were closely associated with helminths, and a further 245 from blackhead and thus probably indirectly due to helminths.

Syngamus trachea appears to be the most important species as a cause of death for it was found in 431 birds and seemed to be responsible for 281 casualties. These figures are probably too low however, and not a true index of the incidence for the infested birds are easily recognisable and many are not sent in for examination. The youngest infestation was seen in an 11-day old chick which had many larvae in the lungs causing typical Syngamus pneumonia : the oldest was a wild hen, nearly 7 years old. This bird had been ringed and tabbed in its first year and, when finally caught, not only were 7 pairs of worms present, but there was also a severe *Histomonas* infection. It was killed *in extremis*. The highest number found in a partridge was 31 pairs.

Young poults are highly susceptible but no age is immune, for many birds which are shot in the ordinary way, are found to carry worms. On one estate in Hampshire, after a big shoot, 60 per cent. of the young birds in the bag were infested with up to 5 pairs of worms, while 22 per cent. of the older birds, i.e. those more than 15 months old, had either 1 or 2 pairs. The distribution of *S. trachea* is patchy. Adult partridges seem to be frequently infested in North Hampshire and in some parts of Norfolk, while adjoining estates remain comparatively free. The host-parasite relationship between partridge and gapeworm shows a number of peculiarities, though there is little difference between infestations among cocks and hens. Adult partridges rarely "gape" even when heavily infested with worms and their reaction is limited to a frequent shake of the head, accompanied by a low pitched snicking noise. Infested birds are very sensitive to handling and will usually die within a few seconds of being picked up. However they will live quite happily with heavy infestations in the wild state and will fly fairly normally over the guns. They easily tire however and soon drop behind the rest of the covey and show some distress when they alight on the ground. Worms do not cause any local irritation, with formation of fibrous nodules, but a good deal of pus is always present.

Trichostrongylus tenuis is another important parasite and in this group of partridges seemed to have been associated with 115 deaths out of a total of 281 infestations. There was no major epizootic during the years under review, but there were several local outbreaks and a number of birds living under artificial game farm conditions

were affected. Reservoir birds, often with very few worms, are important as they contaminate the ground with eggs, so that an epizootic may occur when the right physical and ecological conditions coincide. They are particularly important on game farms and rearing fields where the concentration of birds is high and the amount of land available is limited. Most of the casualties recorded have been from such places. Because of increased interest in partridge rearing as a commercial proposition, this worm is one of those which is bringing about a change in the pattern of disease in this host. It could constitute one of the major hazards of artificial propagation and, as will be seen in the section devoted to pheasants, may cause trouble in that host also.

It has been noticed that trichostrongylosis tends to increase in the spring when the temperature rises high enough to allow the development of eggs deposited during the colder months and when there is plenty of moisture to prevent dessication of the hatched larvae. Further, it is not unreasonable to suppose that the strain of pairing and egg laying may result in some loss of resistance, making infestation easier. The spring rise is actually seen first in cock birds, usually about the middle of February and only appears in hens a month later. The spring food of wild birds consists almost entirely of grass, clovers and other leaves, on which the infective larvae may be lying dormant : the birds thus probably pick up more larvae at this time of the year. A spring epizootic is often followed by a minor one in the autumn, the cause of which is not so obvious but which may be due, in part, to the large number of adolescent susceptible birds.

As with *Syngamus*, *T. tenuis* shows a patchy distribution among partridges. There is for instance, a small area in Norfolk where many partridges always carry a few worms, yet the numbers do not rise to disease levels and partridges on adjoining estates rarely have any.

Other helminths are met with during post-mortem examinations, but are less frequently a cause of loss. *Capillaria* spp. are potential pathogens, but in this series, only *C. cadovulvata* has actually caused death. There were 69 cases of infestation, of which 2 were fatal. One of these has already been described (Clapham, 1949), and the other case followed the same lines. Worms had invaded the jejunum causing inflammation and fibrotic changes.

Heterakis spp. are important helminths. There were 380 cases, 245 of which were associated with fatal *Histomonas meleagridis* infestation and blackhead, 2 with ulcerative typhlitis and only 133 appeared to be non-pathogenic. *H. gallinarum* was the species involved in most cases but *H. isolonche* was present in 92 birds.

The prevalence of blackhead has increased enormously during the last 5 years and has coincided with the artificial production of birds on game farms. It has also increased in the semi-domesticated partridges which live around farms where poultry roam. A surprisingly high percentage of poultry—up to 30 per cent in some areas—have been found to carry *Histomonas* and as such, are dangerous reservoirs which contaminate the pastures grazed by partridges.

The youngest blackhead casualty was a 12-day old penned chick and the oldest was the near 7-year old wild hen mentioned under *Syngamus*. Infected birds begin to show signs of disease 8 days after infection and few partridges live longer than 14 days. Both *H. gallinarum* and *H. isolonche* appear to be possible vectors of *Histomonas* as both have been found in diseased birds.

Ascaridia galli has been found twice ; *Brachylaemus commutatus* once. Cestodes, species of *Hymenolepis* and *Raillietina* were found in 102 birds but none were fatal.

Pheasant (*Phasianus colchicus*)

A total of 3,830 pheasants was examined but this included 767 chicks of little helminthological interest, being under the age of 2 weeks. There were however 3 exceptions which will be mentioned specifically later. Many of the birds were hand reared for a large number of pheasants are hatched under bantam hens or in incubators and then reared under artificial conditions. The data obtained therefore are not necessarily typical of the wild population. But as pheasant rearing is quite an important industry, it is useful to know the main hazards in both natural and artificial environments. As with partridges, helminths are important and 41·72 per cent. of these birds carried one or more species. There were 400 casualties which seemed to be associated with helminth infestation and a further 60 from blackhead, carried by *Heterakis* spp.

S. trachea appears to be the most important parasite, accounting for 477 infestations of which 245 were fatal. As mentioned under the section concerned with partridges, this is certainly not a true index of its prevalence however, for keepers do not send in affected birds for examination. There were a number of interesting cases which can be mentioned. In one case, 3 poults aged 9 weeks which had been taken off the rearing field into the woods 15 days earlier, were sent in as representatives of many that had been found dead. Examination showed that there were 158, 132 and 146 pairs of worms respectively in the trachea while the lungs and bronchi were full of worms unable to find a site for attachment. There were also less well developed

worms in the lungs, which may have been younger or may have been retarded by the weight of parasites present. Those in the trachea were small but mature and gravid : the birds must have picked these massive infestations within 36 hours of being released.

A game farm bird, aged 10 weeks, was found to be carrying 723 pairs of worms in the trachea. They formed a solid core and there were more in the main bronchi. Even more interesting were 15 wild chicks which had died of *Syngamus* infestation. Of these, 10 birds had only larval worms in the lungs and had died of typical Syngamus pneumonia : one of these birds was not more than 10 days old. The other 9 were between 2–3 weeks. The remaining 5 birds had worms in the trachea and 2 of them were estimated to be 13–14 days old.

Adult pheasants are occasionally infested, especially the hens which appear to lose much of their resistance at the breeding season. As a result, there may be some losses in the laying pens. The adult cock is not immune for a number have carried appreciable infestations —one, indeed, had 99 pairs of worms. Sub-lethal chronic infestations seem to be comparatively rare for only occasionally are worms found in birds that have been shot in the usual way, though nodules indicative of previous infestation, are quite common.

T. tenuis is not generally considered a normal parasite of pheasants but 15 cases, 3 of them fatal, have been seen in this series of birds. They were all penned game farm stock and had presumably picked up the larvae from pens previously grazed by partridges. The parasite could become troublesome on game farms unless a careful rotational grazing system is adopted.

Heterakis isolonche is a species found commonly in wild pheasants. It was diagnosed in 304 wild birds and was associated with ulcerative typhlitis, severe enough to cause death, on 148 occasions. But there were also 102 cases of *H. gallinarum* infestation among penned stock, none of which had any typhlitis. Among the same stock 60 deaths had been caused by blackhead and 23 by typhlitis of unknown origin, though a few adult worms, either *H. gallinarum* or *H. isolonche*, were found in each bird. There was no severe larval invasion of the tissues however.

These infestations were not spread evenly over the years. In 1948, large numbers of infested birds were examined and some of them carried considerable numbers of worms, up to 1,008, but tissue damage was slight. This was followed by a period of low frequency until 1955 when the numbers in both wild and penned stock began to increase. This continued in 1956 when most of the cases of ulcerative typhlitis occurred. Numbers of affected birds and of worms carried,

decreased appreciably in the following year and in 1958 were down to normal level. No particular reasons could be given for this sudden peak of disease and later decrease.

Other helminths are less important. *Capillaria cadovulvata* occurred in 72 young birds, all game farm stock and 3 cases were fatal. *C. retusa* occurred in the caeca of 17 birds but was not associated with sickness or real disease. *Ascaridia galli* was present in 9 birds and there were 119 cases of cestode infestation, *Davainea proglottina*, *Hymenolepis* spp. and *Raillietina* spp. being identified. There was a single case of nodular enteritis due to *R. cesticillus*. The only trematode was *Brachylaemus commutatus* which occurred in 2 birds.

Summary

1. Helminth parasites may be important causes of disease and death among game birds, both among penned stock and in the field. *S. trachea* is probably the most important, but species of *Heterakis* follow closely, having both a direct and an indirect effect. *T. tenuis* must be considered as potentially important and may be responsible for much loss when the right ecological factors are present.

2. It is clear that game farmers will need to pay careful attention to methods of controlling the spread of helminths : in particular, to prevent the spread of *T. tenuis*, which can build up quickly to epizootic proportions on restricted areas where the bird population is high. Pheasants are potential hosts of this species and the death rate could easily be serious under careless management.

3. The presence of poultry near or with penned birds is fraught with danger as many helminths have a wide host distribution among related gallinaceous birds. Thus species of *Capillaria* and *Heterakis* which normally cause little trouble among poultry, can cause death and disease among game birds.

4. The few wild chicks examined were interesting. There is a heavy loss of wild chicks but only rarely are dead ones seen so that little is known about the cause of this loss. Adverse weather conditions and predators no doubt are important, but it is obvious from the few cases reported above, that *S. trachea* and *H. isolonche* may also be causes of considerable importance.

5. Many old birds are carriers of parasites and contaminate the ground with ova. Thus young stock with no resistance may well pick up lethal infestations, if they are allowed to graze land previously used by old birds.

Reference

Clapham, P. A., 1949.—"On *Capillaria cadovulvata*, pathogenic to *Perdix perdix*." *J. Helminth.*, **23**, 69–70. (W.L. 11224c).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 41–52.

On the Early Stages of *Fasciola hepatica* Penetrating into the Liver of an Experimental Host, the Mouse: a Histological Picture

By Ben Dawes, D.Sc.
King's College, University of London

The route taken by *Fasciola hepatica* to the final location in the definitive host is a matter which has caused some controversy. The opinion of R. Leuckart was that young forms in the intestines migrate along the common bile duct to the liver but this is erroneous, although it is the route taken by *Clonorchis sinensis*, as Vogel (1934) showed. Lutz (1893) and other investigators suggested that the young flukes travel by way of the portal vein to the liver, but this and also the belief that passage is by way of the lymphatic system was refuted by the work of Sinitsin (1914), Shirai (1927), Susuki (1931), Shaw (1932), Vogel (1934) and Schumacher (1938). These investigators proved that in various natural and experimental hosts the young flukes pierce the intestines, traverse the abdominal cavity, penetrate the liver capsule and wander through the liver to the bile ducts, where maturity is reached. Convincing as is this evidence of migration, nobody has ever produced a satisfying histological account of the passage of young flukes through the liver. Following the classical study already mentioned, Schumacher (1956) fed a single guineapig with 1,000 cysts of *F. hepatica* and killed the animal 64 hours later to remove and section the liver. The analysis of this material, which included 5 photomicrographs, provided only a slight and somewhat tantalising picture of early migration through the liver. The aim of the writer is to amplify this picture and especially to note how the young flukes exist in relation to hepatic tissue, at a time when damage to the liver is not so considerable as to mask the real situation, as it is likely to do in later stages of fascioliasis. The importance of such a study as this is obvious, because the principal characters depicted are the young flukes which are able to resist the action of all known drugs used for the eradication of adult forms resident in the bile ducts.

Methods and Acknowledgments

The writer has produced experimental fascioliasis in rabbits, rats and mice. Apparently, other investigators have not used rats, although they are suitable hosts. The mouse was chosen to serve as host, however, because the enormous task of serialising sections of an infected liver is lessened by the use of a smaller host. Shirai (1927) made use of mice as well as other hosts and Taylor and Parfitt (1957) prefer mice for testing the infectivity of encysted metacercariae, although they claim that a mouse is killed by a single fluke and that mice do not survive more than 6 weeks after infection. Mice are unsuitable for chemotherapeutic work as the flukes leave the liver eventually and return to the abdominal cavity, but this is true of other and larger hosts also and it is not a serious objection when only the early stages of entry are considered.

Mice were given cysts orally by means of a suitably small pipette provided with a pliable extension of tubular polythene which would reach to the stomach. For short experiments about 100 cysts were given, but in longer experiments this number was reduced to 50 and sometimes 10–20. About 50 experiments gave a fairly complete picture of fascioliasis, but no more than about 20 were necessary to cover the early stages of penetration into the liver. A note was kept of the numbers of cysts given, but it was rarely possible to determine the number of flukes recovered. The method of dealing with liver infections was to fix some liver lobes in 10% formol saline and to use the remaining portion of the liver for the recovery of flukes. After sufficient sectioned material had been assembled there was always a reserve of fixed material in hand. Infected hosts were killed at daily intervals during the first 8 days and then after 11, 14, 17 and 28 days, but what is stated refers particularly to the first 8 days or so during an infection. To describe the manner in which cysts were obtained is not necessary. My technician maintained cultures of infected *Limnaea truncatula* which provided some of the cysts used, and others were provided by the kindness of Dr. S. B. Kendall and Mr. J. W. Parfitt of Weybridge, to whom my thanks are now expressed. My personal technician, Mr. John Wells, did not spare himself in producing excellent sections ; he richly deserves my appreciation and thanks. I would like also to thank Mr. K. W. Iles, A.R.P.S., Department of Scientific Photography, Charing Cross Medical School, London, W.C.2 for equally excellent photographic assistance and also to acknowledge gratefully financial assistance from the Agricultural Research Council.

EXCYSTMENT OF METACERCARIAE

This subject was not part of the writer's programme, but mice were sometimes killed 2–3 hours after cysts had been fed, in a quest for young forms in the intestines. Free forms were never found in this rather casual way,* but cysts were recovered, and excystment was witnessed. At the time cysts are fed, they are always examined, and the contained larvae are often seen. The metacercaria flattens itself against the inside of the inner cyst, seeming to fill it, giving a view of the main excretory vessels side by side. Cysts which are found in the intestines after 2–3 hours may appear thus, but in some instances the larva has been activated and then moves about freely, extending and contracting the body in lively motion. Eventually, it attaches itself to the broader part of the cyst by the oral sucker and for some time may remain so attached, but at the point covered by the mouth a hole subsequently appears. It is hardly large enough, but the young fluke squeezes through it with considerable effort. Delicate fibrils can be seen at the margin of the opening; they are broken at slightly different levels and they protrude slightly from the opening, giving it a microscopically ragged edge. The empty cyst sometimes contains droplets of the material, formerly seen in the excretory vessels but now absent; these must be expressed as the young form emerges from the cyst. During emergence, the body is pinched in the middle to give it an hour-glass shape (Pl. 1, A).

EXCYSTMENT IN THE ABDOMINAL CAVITY OF THE HOST

In order to test the possibility that activation of the metacercaria and excystment by perforation of the cyst are independent of digestive processes in the alimentary canal, 30 cysts were administered to each of 2 mice by intra-peritoneal injection, along with antibiotics, care being taken that the cysts should not enter the viscera. The mice were killed after 5 and 6 days respectively and about one half of the cysts were recovered in each case. Some of the cysts contained metacercariae, others were empty, but all had been subjected to an intense foreign body reaction and were in process of phagocytosis. In some instances leucocytes had penetrated into the cysts and were attacking the larvae. Nevertheless, in each animal 3 active young flukes were recovered from the abdominal cavity. The 6 flukes were fixed, stained and mounted, and they gave the following measurements: length, 0·18–0·23 mm.; breadth, 0·10–0·12 mm.; diameters of oral and ventral suckers, 0·034–0·042 mm. and 0·031–0·045 mm. respectively. All the 6 flukes

* Such forms have since been recovered.

are smaller than the flukes recovered 3 days after oral administration of cysts to mice. The experiment should be repeated but, in the circumstances, the result is categoric. The cysts may have been in contact with enzymes, of course, but not enzymes of the alimentary system, which cannot be regarded as essential either for the activation of metacercaria or their emergence from the cysts. The possibility that activation of the larvae occurs in water or in diluted bile when cysts are maintained at 37°C. was also tested by *in vitro* experiments, but the results were negative and there for the time being the matter rests.

Excystment in a "Wrong" Host

If metacercariae can be activated to excyst in the wrong location, they might also excyst in a "wrong" host. To test this possibility, 6 chicks about one week old were given 50 cysts each orally. Two of the birds were killed 3 days later and the others 7, 17, 25 and 34 days from the time cysts were administered. None of the birds showed any lesions on the liver, although in a 3 day experiment there was a slight vascular swelling on the liver of the bird. In this experiment 6 active young flukes were recovered from the abdominal cavity and in the other 3 day experiment 2 similar flukes. The 17 day experiment also provided a single active fluke, evidently larger than the others and likewise found in the abdominal cavity. All 9 flukes were fixed, stained, mounted and measured, giving the following results :—

Number of days	*Number of flukes*	*Length (mm.)*	*Breadth (mm.)*	*Diameter of oral sucker (mm.)*	*Diameter of ventral sucker (mm.)*
3	8	0·18–0·24	0·12–0·16	0·040	0·045
17	1	0·47	0·35	0·08	0·08

The younger flukes are smaller than any which were recovered in 3 days after oral administration of cysts to mice and about the same size as those recovered after intraperitoneal administration of cysts. The older fluke was appreciably larger and had (during 14 days) doubled its length and breadth, but was much smaller than a 17 day fluke from the liver of a mouse, which is more than 3 mm. long. The experiments were not continued, although the astonishing thought arises that table birds might help in the control of fascioliasis. It is evident, making the least of these results, that a young fluke can maintain itself and even grow a little by browsing on the viscera of even a "wrong" host.

FREE FLUKES IN THE ABDOMINAL CAVITY

A number of 3-day experiments were carried out which provided assemblages of young flukes in the abdominal cavity of the mouse. In a typical experiment, when 100 cysts were administered, 25 free flukes were recovered from the abdominal surface of the intestines, 2 flukes were seen creeping about on the surface of the liver and several flukes had already entered the liver. The free flukes are lively and very extensile and contractile, size being decidedly variable, but when the flukes are fixed they become rounded off to a more or less constant shape. The measurements of a random sample of 20 stained and mounted 3-day flukes are as follows :—

Length (*mm.*)	*Maximum Breadth* (*mm.*)	*Diameters of suckers* (*mm.*)	
		Oral	*Ventral*
0·25–0·35	0·16–0·22	0·04–0·06	0·05–0·065

In 17 out of 20 specimens, the major part of the ventral sucker is situated in the posterior half of the body, and in the remaining 3 the sucker is almost exactly at the mid-body level. The flukes have a typical pharynx, short oesophagus and two simple caeca with very slight lateral lobes. A dumb-bell shaped dense aggregate of embryonic cells extending into the posterior region from a level just in front of the ventral sucker is the rudiment of the reproductive system.

In 3-day experiments the liver usually shows several microscopic lesions in the form of minute holes with ragged edges surrounded by either a bubble-like area of greyish colour or an elongate tract of tissue having the same greyish appearance. In each of 2 other experiments in which lesions were seen a single young fluke was seen to be half buried in the liver, twitching the posterior end of the body excitedly as it entered the liver. Experiments extending over more than 3 days revealed more extensive fluke tracks, generally having the same greyish colour, but sometimes reddish streaks also. In all experiments lasting 3–8 days free flukes were found in the abdominal cavity, though only in small numbers. No liver lesions were found in shorter experiments, but free flukes were found in the abdominal cavity within 24 hours of feeding cysts to the host. Evidently, flukes may wander about in the abdominal cavity of the mouse for at least a week before entering the liver. They may indeed be found after much longer periods of 14–28 days, although such forms are notably larger and indistinguishable

from flukes recovered at corresponding times from the liver : they are forms which have inadvertently made their exit from the liver.

Penetration into the Liver of the Host

Young flukes enter the liver of the mouse when about 0·2 mm. long. Entry was never effected in less than 3 days from the time cysts were taken, but the fact that some young flukes had penetrated deeply into the liver within 3 days suggests that entry may be effected in 2 days, and possibly in 1 day. In one instance of partial entry (Pl. 1, B) the young fluke has penetrated so that both suckers are inside the burrow, the breadth of which is only a little larger than the breadth of the fluke. The mouth of the opening is slightly narrowed and has a kind of flap attached at one side, which seems to indicate that the original hole was smaller than the opening now seen and that the entering fluke undercut the liver capsule slightly as it made the burrow. Here also is the first indication that burrowing takes place along an arc-like course, the anterior end of the fluke being flexed ventrally during progression and this is presumed to be the reason why the early burrows approach the surface of the liver to provide superficial tracks. The burrow contains some cellular debris—including ruptured hepatic cells, pyknotic nuclei, leucocytes and some erythrocytes. The debris is smoothed down against the side of the burrow by movements of the fluke, and some of it is attached to the flap at the mouth of the burrow. As the burrow is deepened (Pl. 1, C) there is considerably more debris of the same kind and this is more abundant near the dorsal side of the fluke than on the opposite side. The anterior end of the fluke is directed towards hepatic cells and most of the broken tissue and pyknotic nuclei must be swept back over the anterior extremity to be smoothed down in the picture seen. In making a burrow just sufficient to lodge its body the fluke must have broken down several hundred hepatic cells, together with sinusoids and cells of the reticular endothelial system. There is some bleeding into the burrow and erythrocytes can be seen about the body of the fluke.

Transverse sections passing through a fluke of the same age which is deeply embedded in the liver provide a better measure of damaged tissue at the level of the pharynx (Pl. 2, A) and the ventral sucker (Pl. 2, B) ; the greater heaping up of debris on the dorsal side of the fluke is more clearly seen and the smoothing down of the debris also. The caeca of this fluke contain cellular debris and erythrocytes as well, but the latter are not abundant, many being

incorporated in the debris and also lying free in the lumen of the burrow. Incidentally, the fluke is finely spinous, but the stippled effect seen in the obliquely cut integument in Pl. 2, A, is due to longitudinal muscle fibrils, which are transversely cut and more diagrammatic in Pl. 2, B. The advancing anterior end of yet another 3-day fluke is seen in Pl. 3, A, to have a beak-like rim which, allowing for slight shrinkage, is pinching off a fragment of hepatic tissue comprising 3 hepatic cells with pyknotic nuclei and several cells of the R.E.S. Behind the oral sucker, the burrow is more expanded and in the relatively lateral plane depicted, many leucocytes (lymphocytes and especially band-type neutrophils and polymorphs) can be seen amidst the cytoplasmic and nuclear debris. Another section passing through the same fluke (Pl. 3, B) clearly shows how the anterior end of the body is directed towards normal hepatic tissue.

Infections older than 3 days show progressively increasing liver damage due to growth of the fluke as well as continued burrowing. At 3–4 days from the time cysts were taken, the fluke is about 0·33 mm. long; at 6 days its length is about 0·48 mm. and at 8 days about 0·8 mm. The following observations were made on specimens of about this age in the liver, but apply to older flukes up to about 11 days, when the length is 1·2–1·6 mm. The older stages provided flukes 3·6 mm. long at 17 days, up to 3·8 mm. long at 20 days and 4·85 mm. long at 28 days, by which time the typical leaf-like form of the adult has been attained in miniature and the reproductive organs and their ducts are clearly recognisable. The flukes older than 3 days have a burrow which comprises a series of ovoid chambers about as long as the worm with intervening rather narrower connections. The series of connected chambers show a slightly zig-zag arrangement, which suggest that the fluke re-orientates its body periodically, as would seem to be necessary when we recall the arc-like form of the early burrow, a region of especially compressed hepatic cells marking the point at which direction was changed slightly. After 6 days, the burrow may extend nearly across the liver, but seldom in one plane, sections generally showing some of the chambers but not all of them. This is illustrated in Pl. 4: the fluke has moved forward into fresh hepatic tissue after slight re-orientation, leaving behind it in the previously occupied chamber debris formed in the excavation of the most recent chamber, together with erythrocytes which have seeped into the burrow and some leucocytes which have invaded it. A coagulum is also seen in the figures; this stains much like the cytoplasm of the liver cells and is probably derived from it. Another characteristic of these chambers making up the burrow

is signs of compression in the hepatic cells just external to broken cells in the wall of the burrow. These compressed cells, which are most numerous in the narrow regions between successive chambers, show a striking staining reaction which is shared by only one other tissue seen in sections of infected liver—the caecal epithelium of the fluke. No matter what stains are used, this characteristic reaction is seen, and it is possibly an indication of alteration produced by secretions of the gut of the fluke. No doubt the young fluke empties its gut from time to time, as it is seen to do *in vitro*, so that the burrow will contain any digestive enzymes which are produced by the fluke. The caeca of this fluke contain hepatic cell cytoplasm, pyknotic nuclei and erythrocytes (Pl. 4, B) and invading leucocytes are migrating into the burrow from the portal vessel indicated below and on the left.

In many instances the oral sucker has been seen biting deeply into a mass of hepatic cells, looking like pincers in section and in Pl. 5, A, a large chunk of cytoplasm is seen within the cavity of this sucker. To be noted also, particularly in the enlarged anterior end of this fluke (Pl. 5, B), is the very dense aggregate of nuclei belonging to the oral sucker and the "neck" of the body, indicating tremendous rigidity in this part of the body. The anterior end of the fluke is a rigid probe terminating in a powerful grasping organ which is capable of inflicting serious damage to the hepatic cells by purely mechanical means. The posture of the fluke, the anterior region flexed and the dorsal region pressed against the wall of the burrow, supports this view. Some of the compression in the hepatic cells lining the burrow may be related to the mechanical effort, though it is more likely to indicate pressure inside the burrow. A similar effect is seen in liver abscesses. The tissue feeding preference of the young fluke is illustrated by the caecal contents seen in sections at this time (Pl. 6, A), the food mass consisting of a mixture of hepatic cell cytoplasm, pyknotic nuclei of hepatic cells and Kupfer cells, leucocytes and some erythrocytes. At any rate, the fluke is not feeding on blood. This example, more highly magnified (Pl. 6, B), shows an almost complete hepatic cell including the nucleus in the caecum on the right.

It is inevitable that in its wanderings through the liver, the young fluke will from time to time encounter larger blood vessels and were it truly a blood-feeder it could be expected then to demonstrate a preference for a blood meal. Several instances of very close approach to fairly large portal vessels are seen in the writer's sections and two of them are illustrated in Pl. 7. In each instance, the advancing anterior end of the fluke is pressing into normal

hepatic tissue after having turned away from a region (marked x) in which hepatic cells have been removed or macerated right up to the wall of the vessel, which itself is slightly damaged but has not ruptured. The shape of the ultimate chamber of the burrow, i.e., that in which the fluke is lying, suggests that this is not due to the zig-zag mode of progression of the fluke, but is a change of direction of a more pronounced kind than is usually seen. The writer believes it to be a movement determined by the fluke's preference for hepatic cells as food. At any rate we could not affirm that the fluke is showing any tendency to seek blood. Young flukes could at any time obtain blood by merely turning about in the burrow, the narrowness of which does not preclude this possibility. The whole action of the young fluke in many graphical examples seems to be to keep the oral sucker pressing into a mass of hepatic cells (Pl. 8, A). Flukes which have been burrowing in the liver for several days are generally deeply imbedded in normal hepatic tissue, and sometimes little damage is done to the hepatic cells immediately around the fluke (Pl. 8, B), debris having been forced back into the penultimate chamber as the animal progressed forward.

DISCUSSION

Sinitsin did not comment about excystment, but Susuki (1931) placed cysts in artificial digestive juices and when discussing the result stated "the larva must be active in the capsule and the pressure inside the capsule must be high enough if the larva is to penetrate the wall of the capsule". Vogel placed 100 cysts in fresh intestinal juice from a dog, kept them at a temperature of 37°C. and recovered 25 young flukes after $1\frac{1}{4}$ hours, 65 after $2\frac{1}{4}$ hours. In a short note in parentheses he wrote : "die Cystenhülle waren nicht verdaut, sondern an ihrer Basis von den Egeln durchbrochen worden". Schumacher (1938) fed 400 cysts to a guineapig and killed the animal 30 minutes later, finding in the stomach unaltered cysts and empty cysts. He wrote : "Leere innere Cystenhüllen von gleichmässig durchsichtigen Struktur und abgeflacht halbkuglichen Form zeigten an der Basis eine kreisförmige Öffnung, durch welche die Cercarie die Hülle verlassen hatte". Farther on, he remarked that the outer cyst is removed in the stomach, often in a single piece and the inner cyst passes on into the small intestines. Then he added : "Dort schlüpt sie (the metacercaria) an der Basis der Cyste, d.h. an der schwächsten Stelle durch eine kleine, runde Öffnung". Hughes (1959) observed that the metacercaria sometimes attaches itself to the cyst, buffeting the wall with its anterior tip and that it may attach by means of the oral sucker. The young fluke emerges anterior end foremost but Hughes did not see the

manner in which it emerged. The factors which control activation of the larva and excystment must be simple, but they are still undetermined. The writer has shown that the digestive enzymes of the gut are not essential, and that raising the temperature of a watery medium to 37°C. will not suffice, whether or not bile is present. It seems likely that the activated larva perforates the cyst by mechanical means coupled with the use of its own digestive secretions; the digestive enzymes of the host seem to have little or no effect on the inner cyst. The problem which requires investigation is the activation of the larva.

The writer has not made any serious effort to determine the earliest appearance of young flukes in the abdominal cavity of the mouse, but has noted that they appear there within 24 hours of the feeding of cysts to the animal. Schumacher (1938) specially concerned himself with this problem, and he found that flukes reach the abdominal cavity in the guineapig in 2 hours, but are still assembling there for 24 hours. The writer found that flukes have penetrated the liver of the mouse by the 3rd day, from which it is inferred that some may enter on the 2nd day after feeding cysts to the animal. Schumacher found that an occasional fluke may penetrate the liver only 8 hours after the host received cysts, but that most forms begin to enter the liver after 48 hours and that some are still entering 3–6 days after cysts were administered. At the end of 6 days about one quarter of the cysts administered have yielded flukes that have entered the liver. Shirai (1927) fed cysts to guineapigs and 3–4 days later found flukes in the abdominal cavity and on the surface of the liver. In his experiment with two guineapigs united "paratiotically" he found liver lesions after 3 days. Shaw (1932) fed 900 cysts to a guineapig and recovered 246 flukes from the abdominal cavity 3 days later, but flukes had not then entered the liver. Evidently, there is much variability in the time taken for young flukes to penetrate the liver. The late-comers which have often spent much time browsing on other organs in the abdominal cavity are of stunted growth and handicapped in this way may give rise to apparently inhibited forms such as are known to remain in the liver parenchyma in single infections after maturing forms have entered the bile ducts, forming a population of flukes able to resist drugs which will destroy mature forms.

Various writers have reported the presence of older flukes in the abdominal cavity of the host, forms which have spent some time in the liver but have inadvertently emerged from it. The writer has found flukes in the body cavity after 17 and 28 days in

the mouse. Schumacher (1938) found such flukes in guineapigs after 23, 24, 34 and 35 days and in sheep after 35 and 56 days. In the matter of size such flukes agree with forms which have never left the liver in corresponding periods. They may leave the liver which has been badly damaged, but the writer has seen a number of instances in which emergence from a little damaged liver is purely accidental; the fluke continues its burrow until it comes to lie close beneath the capsule far from the entrance, and it may then turn aside, but sometimes it ruptures the capsule and may emerge from the opening. Sinitsin (1914) found flukes creeping about over the viscera 4–14 days after cysts were taken, but these were forms which had not entered the liver, because he stated that they could be distinguished from liver dwelling forms of corresponding age by the plump and well nourished state of the latter. In this sort of experiment, however, age (as reckoned by the time which has elapsed since cysts were administered) is a poor criterion of size. In the writer's experiments with mice, the length of the fluke in terms of "days" varies as follows: 0·3 mm. (3–4 days), 0·48 mm. (6 days), 0·8 mm. (8 days), 1·2–1·6 mm. (11 days), 3·6 mm. (17 days), 3·2–3·8 mm. (20 days) and 4·85 mm. (28 days). For Sinitsin's experiments with rabbits the data available are as follows: length: 0·3 mm. (2 days), 0·5 mm. (7 days), 1·0 mm. (14 days) and 4·6 mm. (24 days). For Schumacher's experiments with guineapigs the data are: length: 0·2 mm. (2 hours), 0·3 mm. (3 days), 0·5 mm. (6 days), 3 mm. (24 days) and 4·5 mm. (34 days).

The amount of damage done to the liver will depend at any time on the size of the advancing fluke and when we consider that a fluke can double its length between the 3rd and 8th days, double it again during the next 3 days and still again after 4 more days, the rate of increase in the damage done daily is also considerable. The writer's photomicrographs provide grounds for believing that for most of the period specified the young fluke displays a preference for hepatic cells as an article of diet. As time continues, however, increasing amounts of blood from damaged sinusoids will leak into the fluke burrows and possibly this will influence the fluke's partiality for such a diet. Attempts are being made to decide what the fluke's preferences may be in the later stages up to the attainment of maturity. Meanwhile, what the writer's experiments have disclosed will not be without significance in chemotherapeutic studies of experimental fascioliasis. The most profitable method of controlling the disease by drug treatment is to kill young forms as soon as possible. In the writer's opinion, such drugs as will serve this end should be capable of storage in the hepatic cells on which young forms largely feed.

Summary

1. Excystment of the metacercariae of *Fasciola hepatica* by perforation of the inner cyst was witnessed, but the factors which control activation of the larva remain undetermined.

2. Excystment occurred in two instances when cysts were administered to mice by intra-peritoneal injections, active young flukes being recovered.

3. Excystment occurred when cysts were administered to one week old chicks and free flukes were recovered from the abdominal cavity in three instances, but liver lesions were not observed.

4. By means of photomicrographs an attempt has been made to demonstrate that young flukes feed primarily on hepatic cells and that they show a preference for this diet. Blood is inevitably included in the diet of young flukes, but it is claimed that these are not truly blood feeders.

5. Young flukes recovered from the liver of the mouse range in length from about 0·3 mm. at 3–4 days to 4·85 mm. at 28 days from the time cysts were administered (*per os*). This accords fairly well with data obtained by Sinitsin and by Schumacher in respect of flukes from rabbits and guineapigs respectively.

References

Hughes, D. L., 1959.—"Chemotherapy of experimental *Fasciola hepatica* infections." *Ph.D.Thesis, University of London.*

Lutz, A., 1893.—"Weiteres zur Lebensgeschichte des *Distoma hepaticum*." *Zbl. Bakt.*, **13**, 320–328. (W.L. 23684.)

Schumacher, W., 1938.—"Untersuchungen über den Wanderungsweg und die Entwicklung von *Fasciola hepatica* L. im Endwirt." *Z. Parasitenk.*, **10**, 608–643. (W.L. 23536b.)

————, 1956.—"Untersuchungen über das Eindringen der Jugendformen von *Fasciola hepatica* L. in die Leber des Endwirts." *Ibidem*, **17**, 276–281.

Shaw, J. N., 1932.—"Studies of the liver fluke (*Fasciola hepatica*)." *J. Amer. vet. Ass.*, **81**, N.S. **34**, 76–82. (W.L. 11022.)

Shirai, M., 1927.—"The biological observation on the cysts of *Fasciola hepatica* and the route of migration of young worms in the final host." *Sci. Rep. G. Inst. infect. Dis. Tokyo Univ.*, **6**, 511–523. (W.L. 19989h.)

Sinitsin, D., 1914.—"Neue Tatsachen über die Biologie der *Fasciola hepatica* L." *Zbl. Bakt.*, **74**, 280–285. (W.L. 23684.)

Susuki, S., 1931.—"Researches into the life-history of *Fasciola hepatica* and its distribution in Formosa, especially on determination of the first intermediate host and some experiments with larvae freed from their cysts artificially." *Taiwan Igakkai Zasshi*, **30**, 97–102 (English summary); 1418–1469 (Chinese account). (W.L. 11337.)

Taylor, E. L. and Parfitt, J. W., 1957.—"Mouse test for the infectivity of metacercariae with particular reference to metacercariae in snail faeces." *Trans. Amer. micr. Soc.*, **76**, 327–328. (W.L. 21400.)

Vogel, H., 1934.—"Der Entwicklungszyklus von *Opisthorchis felineus* (Riv.) nebst Bemerkungen über die Systematik und Epidemiologie." *Zoologica, Stuttgart*, **33**, 1–103. (W.L. 23820.)

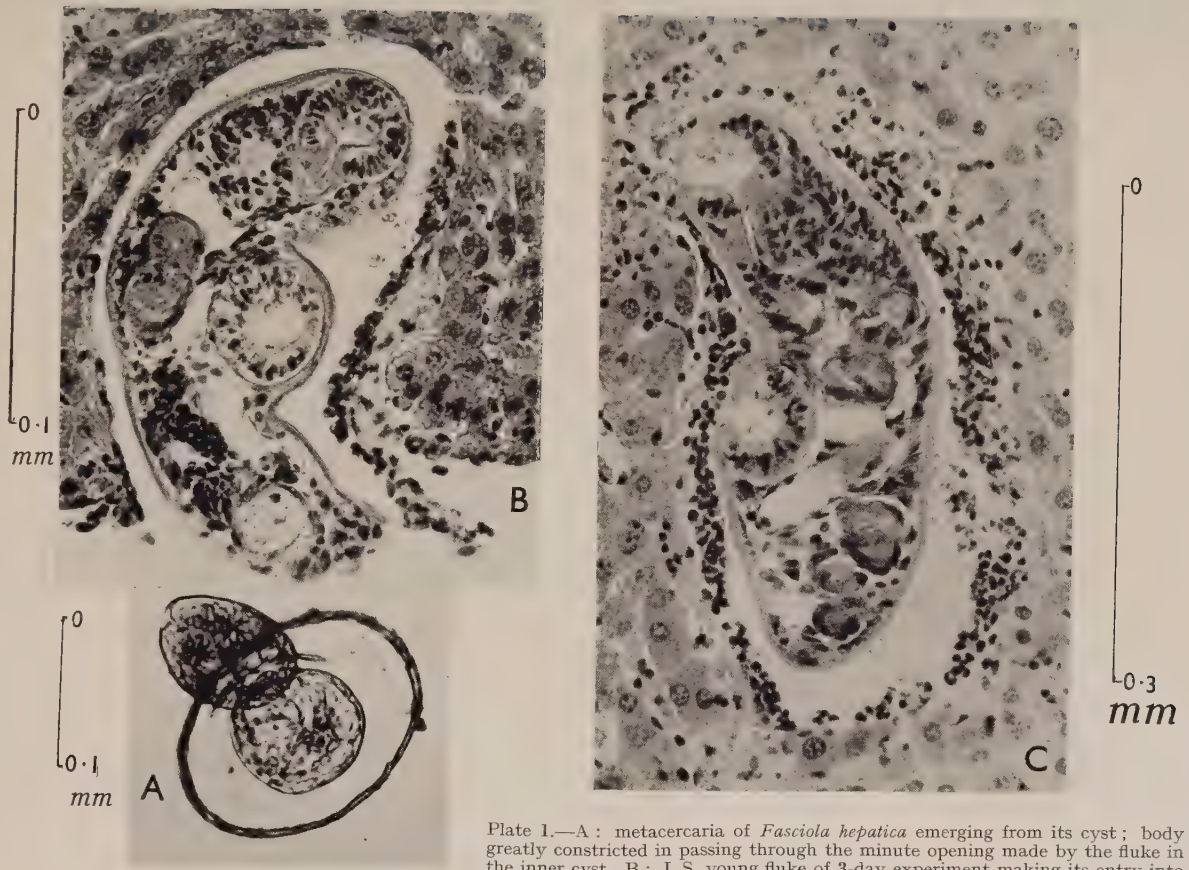


Plate 1.—A : metacercaria of *Fasciola hepatica* emerging from its cyst ; body greatly constricted in passing through the minute opening made by the fluke in the inner cyst. B : L.S. young fluke of 3-day experiment making its entry into the liver. C : L.S. fluke of same age located in an early burrow. Further explanations in the text.

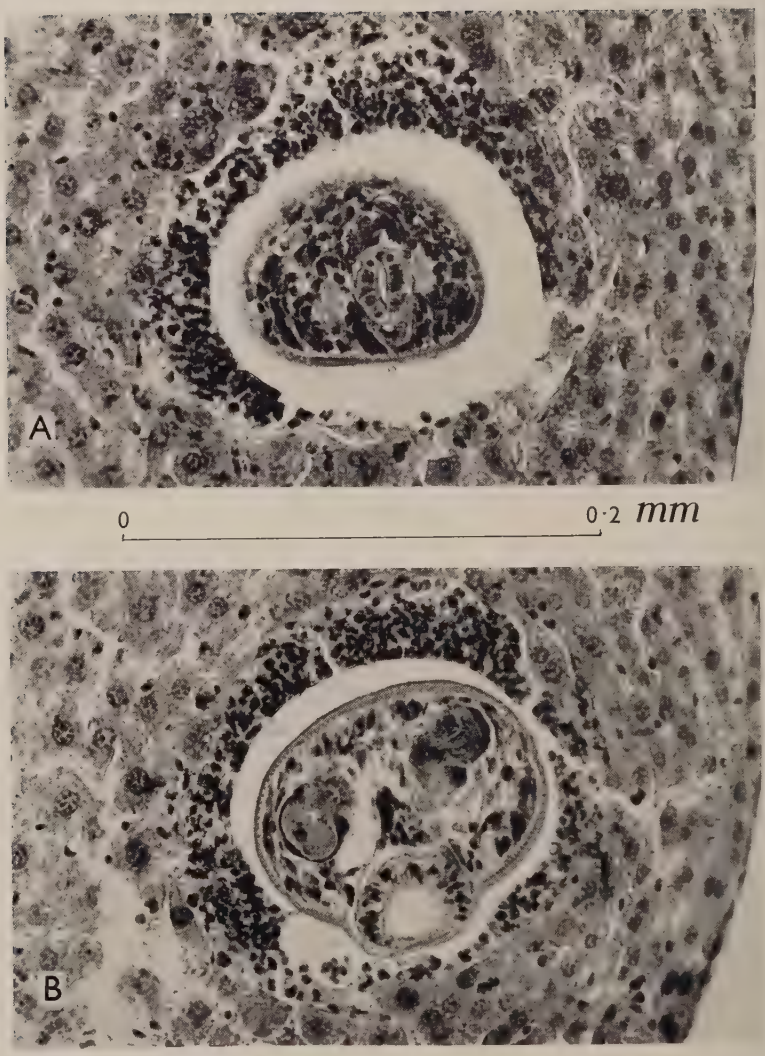


Plate 2.—T.S. young fluke (3-day exp.) located in burrow. A : in region of pharynx. B : in region of ventral sucker. Further explanations in the text.

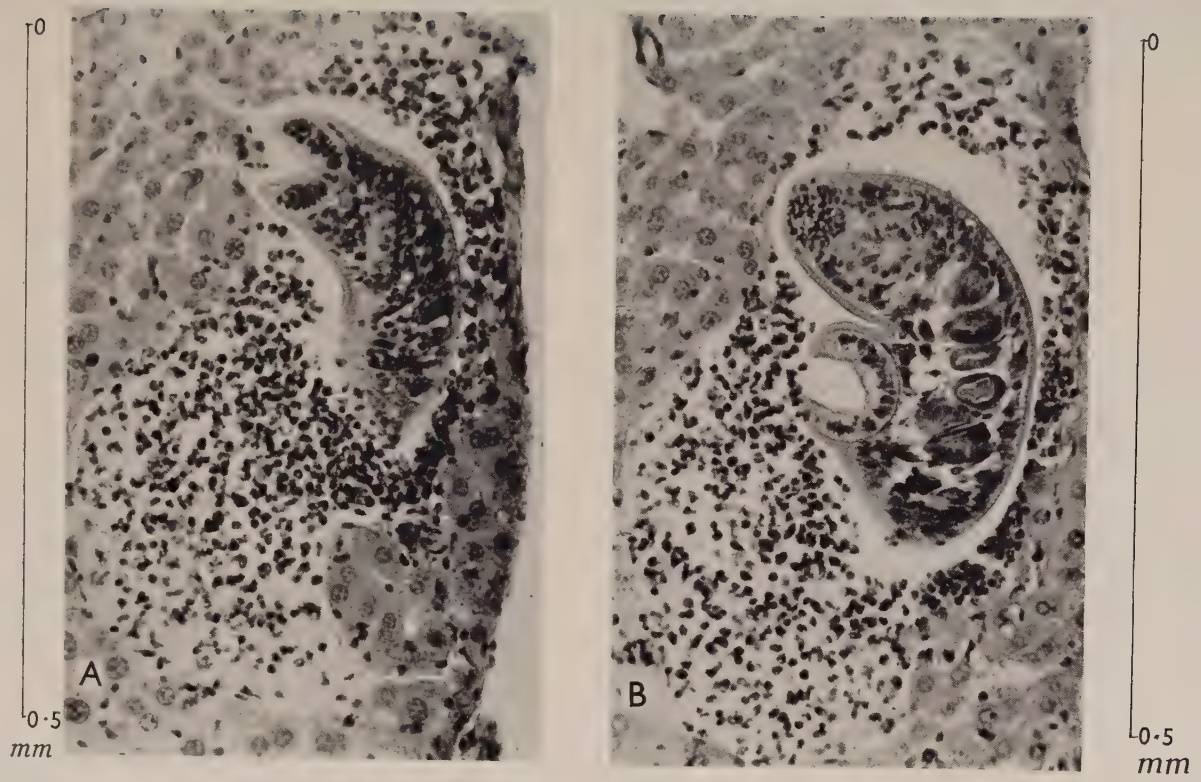


Plate 3.—L.S. young fluke (3-day exp.) in liver. A : in region of oral sucker and "neck". B : in region of pharynx and ventral sucker. Further explanations in the text.

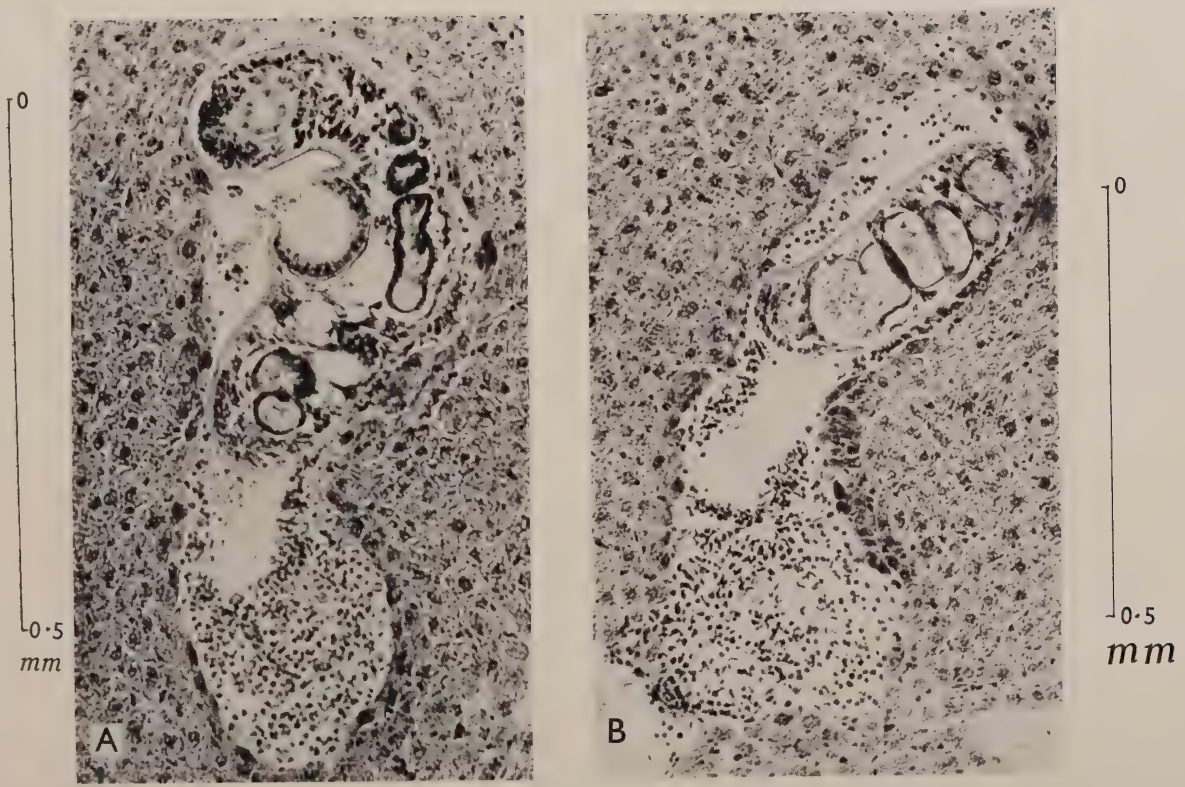


Plate 4.—L.S. fluke in older burrow (6-day exp.). A : in region of pharynx and ventral sucker. B : in region of caeca. Further explanations in the text.

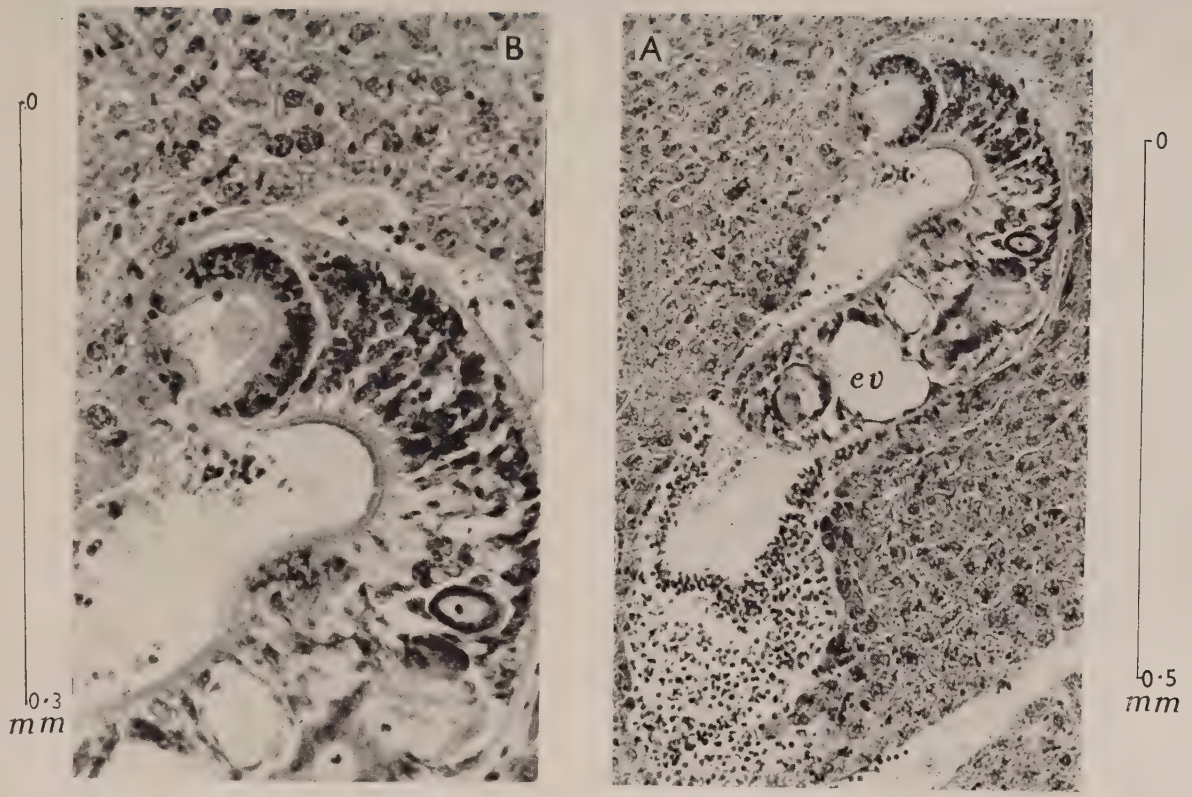


Plate 5.—L.S. fluke in burrow (6-day exp.). A : showing an almost entire fluke which is moving into fresh hepatic tissue. *ev*, excretory vesicle. B : showing, with greater magnification, the anterior end of the body. Further explanations in the text.

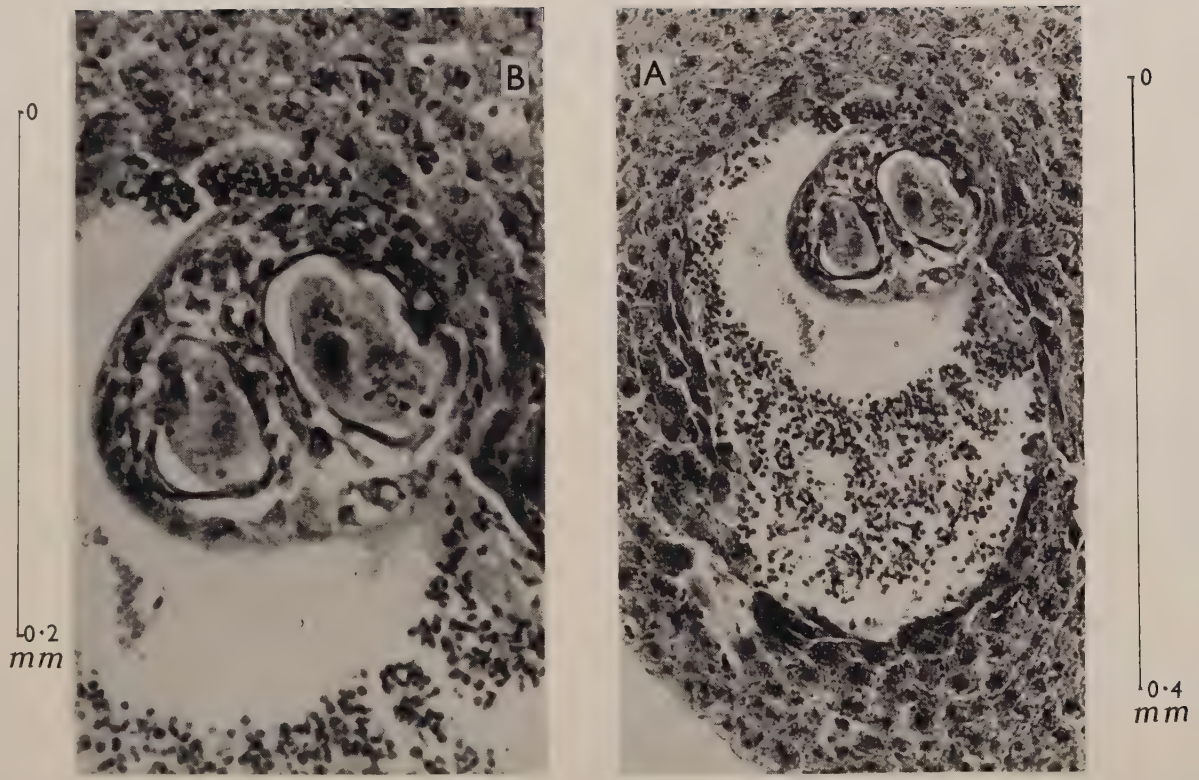


Plate 6.—T.S. fluke (6-day exp.) showing the caecal contents. B : more highly magnified view of fluke shown in A, depicting a hepatic cell in the caecum on the right. Further explanations in the text.

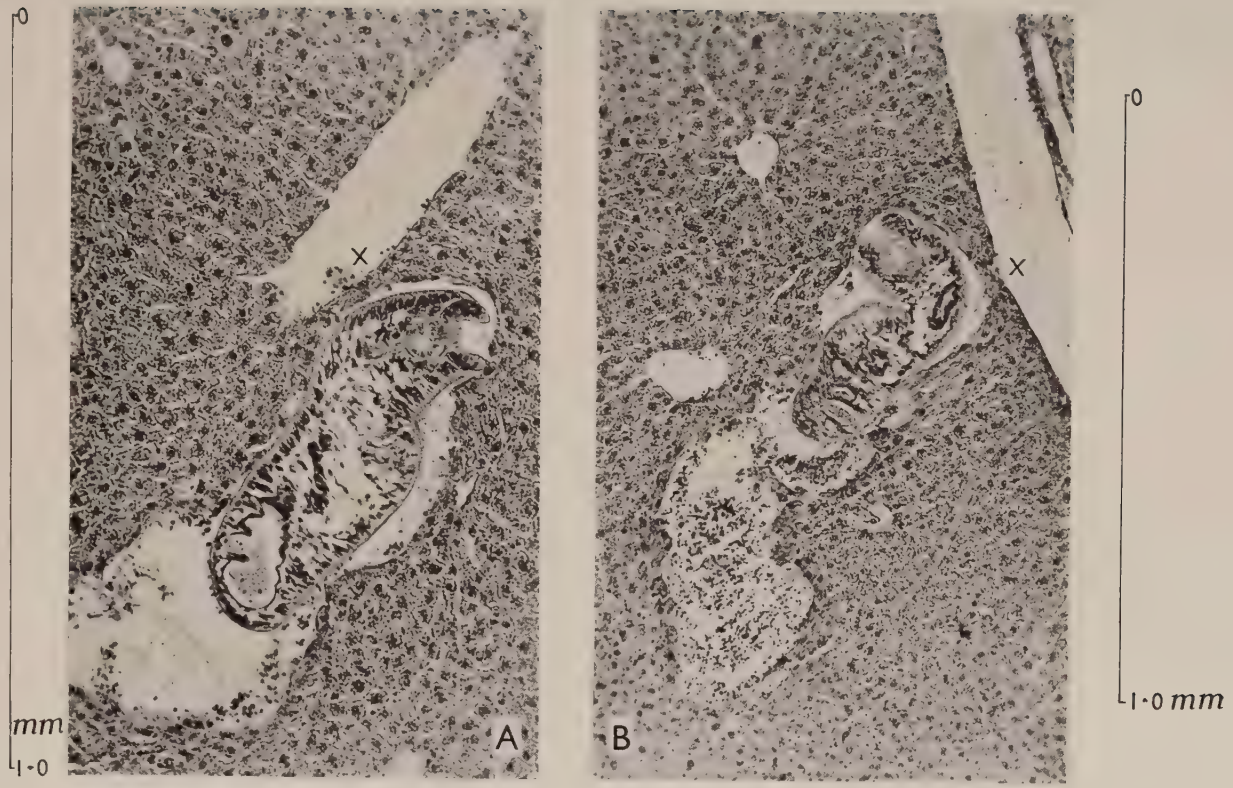


Plate 7.—L.S. two flukes (6-day exp.) each showing "avoidance" of a portal vessel in the liver. Further explanations in the text.

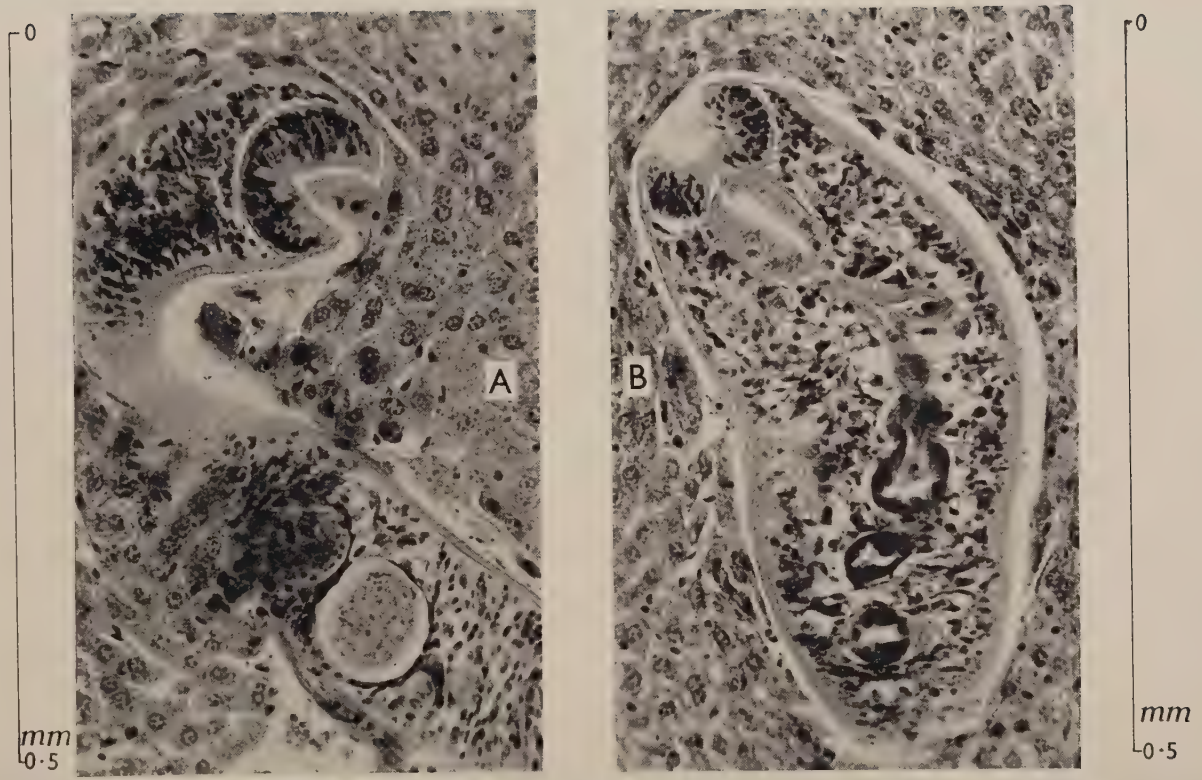


Plate 8.—A : L.S. anterior end of fluke advancing into hepatic tissue. B : almost frontal section of fluke showing location in little altered hepatic tissue (6-day exp.) Further explanations in the text.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 53–62.

On the Morphology and Life History of *Fasciola nyanzae* Leiper, 1910 from the Hippopotamus

By J. A. Dinnik and N. N. Dinnik
East African Veterinary Organization, Muguga, Kenya

A half a century ago, R. T. Leiper described a new species belonging to the genus *Fasciola* from specimens which he collected from hippopotami at the Murchison Falls, on the Victoria Nile in Uganda, in the summer of 1907. This species, *Fasciola nyanzae*, was found in the bile ducts of the liver, and in most instances the specimens were somewhat macerated owing to the length of time that had elapsed before the animal could be recovered from the water. Nevertheless, the characteristic shape of these liver flukes and the restriction of the branched testes to the anterior third of the body made it possible to recognise them as being different from all other species of the genus known at that time.

The second time a number of well preserved specimens of *F. nyanzae* were found by H. G. Jackson among the material collected by R. E. Montgomery in British East Africa. The examination of these specimens enabled Jackson (1921) to add details of their inner morphology to Leiper's original description of the species and to furnish the description with two fine illustrations.

In May, 1958, a collection of parasitic worms was sent by Dr. W. M. Longhurst, a Senior Fulbright Scholar working with the Uganda Game Department, to the East African Veterinary Research Organization for identification. Among the material collected from hippopotami in the Queen Elizabeth National Park, Uganda, three specimens of *F. nyanzae* were found. It was decided to use the opportunity offered by the control shooting of hippopotami in the Park at that time to collect more material of *F. nyanzae* and to attempt to elucidate the life-history of this, the third *Fasciola* species known in Africa. Further specimens of *Fasciola nyanzae* were collected by Dr. Longhurst during his research on the biology of the hippopotamus and by Mr. T. J. Coyle of the Animal Health Research Centre, Uganda, as a result of the dissection of hippopotami shot in the Park. The authors also went to the Park and spent ten days there collecting specimens of liver flukes and their eggs for experimental attempts to study the life history of *F. nyanzae*.

The livers of fifteen hippopotami were dissected and liver flukes were found in the bile ducts of nine of them. The number of flukes recovered was small, from 1 to 5 specimens per animal. Cystic enlargement of the bile ducts and the development of scar tissue round the ducts, with the characteristic appearance of pipe-stems, as is usual in domestic ruminants infested with *F. hepatica* and *F. gigantica*, were not seen. Eggs were obtained from specimens of *F. nyanzae* recovered from two hippopotami and were also collected from the gall bladders of these two animals in order to hatch miracidia for the experimental infection of snails. In addition fresh dung of hippopotami was collected for examination along the shores of the Katwe Bay of Lake Edward and the Kazinga Channel. In seven out of thirty samples of dung the eggs of *Fasciola* were found.

Morphology

Seventeen specimens of *Fasciola nyanzae* from the hippopotami taken at Lake Edward were examined; they measure from 49 to 91 mm. in length and are all sexually mature with eggs in their uteri. The form of the body of these specimens is very characteristic and they might be described as being oblanceolate, narrowly rounded at the posterior end and very shortly and obtusely mucronate at the anterior end. The cephalic cone is well developed and the shoulders are distinct. The greatest width of the body is attained at the level between the ventral sucker and Mehlis' gland, or slightly posterior to this, and it then tapers gradually towards the bluntly pointed posterior end (Fig. 1).

The body is covered with scales which are similar to those on the body of *F. gigantica* in shape, being broad and bluntly edged. On the cone the scales are 43 to 85μ long, increasing in size from the anterior to the basal part, and on the body they vary from 82 to 92μ in length.

The ventral sucker lies at the base of the cephalic cone and its antero-posterior diameter varies from 1·46 to 1·97 mm. The oral sucker measures 1·15 to 1·55 mm. in diameter and the strong pharynx, which is long and oval in shape, varies from 0·90 to 1·30 mm. in length and 0·50 to 0·85 mm. in width. The oesophagus is short, 0·23 to 0·50 mm. long, being 3 to 5 times shorter than the pharynx.

Two main stems of the gut skirt the sides of the ventral sucker and approach one another again at the level of Mehlis' gland; they

continue to run very close together for the rest of their course to the end of the body. In the cone each gut stem has 4 to 6 lateral diverticula which are short, thick and divided into a few short secondary branches. There are a few short unbranched diverticula on the inner sides of the gut in the cone.

Fig. 1.—Six specimens (to the left of the scale) of *Fasciola nyanzae* Leiper recovered from *Hippopotamus amphibius* in Uganda and a single specimen of *Fasciola gigantica* Cobbold recovered from *Bos indicus* in Kenya.

The first extensively ramose lateral diverticula are given off from the gut stems posterior to the ventral sucker. In the area of the uterus and Mehlis' gland these lateral diverticula are directed antero-laterally; at the level of the first testis they are more or less at right angles to the main stems of the gut and posterior to this part of the body they become more and more inclined backwards. Finally, in the last quarter of the body, they run almost parallel to the gut stems. These lateral diverticula vary in length and degree of ramification. The largest ramify into numerous irregular branches whose blind ends either reach the lateral edges of the body or terminate in its lateral field between the other branches.

The branches of the shorter diverticula do not reach the edges of the body and there are also some very short diverticula which merely possess a few short branches or sometimes none at all.

The internal diverticula of the gut stems are fairly numerous, varying in number from 23 to 44 on each side. They are all short and simple, in the form of either a small projection on the inner

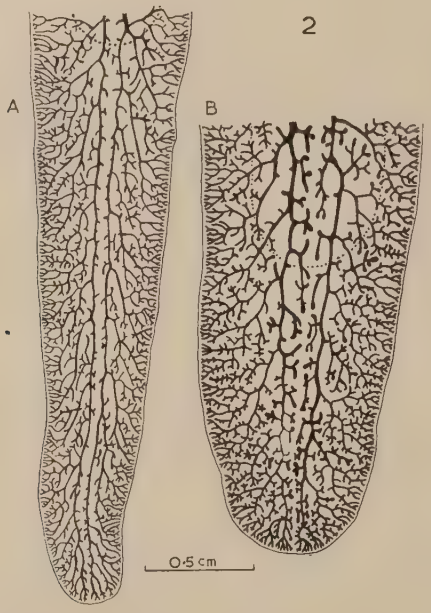


Fig. 2.—The gut ramifications in the posterior part of the body in *Fasciola nyanzae* (A) and *Fasciola gigantica* (B)

side of the gut stem or a short single diverticulum or a short diverticulum divided into two distally ; most of the internal diverticula, however, are unbranched (Fig. 2).

The genital organs resemble closely those in *F. gigantica* but the space occupied by the ovary and the testes in the body is much smaller. The testes lie in the anterior third of the body ; in our specimens the field occupied by the testes varies from one quarter to two fifths of the entire length of the body.

The ovary is on the right in all the specimens except one. In this specimen, close to Mehlis' gland, the ovary is divided into two branches, one of which turns right and the other left, and both form ramifications on the corresponding side of the body.

The vitellaria extend anteriorly to the level of Mehlis' gland or even to the level of the posterior border of the ventral sucker. In no specimens were the yolk glands seen to extend into the shoulders. In the anterior third of the body the vitellaria surround the testes on both sides and do not extend into the field occupied by them. At the posterior margin of the testes the vitellaria from the two sides close up and are spread over the whole width of the body, merely leaving a narrow space free from yolk glands along the midline. Groups of yolk glands lie on both the ventral and the dorsal sides of the gut ramifications in all the specimens we have examined.

The coils of the uterus lie between Mehlis' gland and the ventral sucker and the lateral coils extend slightly anteriorly on both sides of the sucker.

The eggs measured in the specimens mounted in Canada balsam varied between 131 and 160 μ in length and from 75 to 88 μ in breadth. The eggs obtained from fresh specimens of the flukes and from the gall bladder of infested hippopotami were 132 to 150 μ long and 79 to 97 μ wide. Specimens have been lodged in the collection of the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Life History

Along the shores of Lake Edward and the Kazinga Channel *Lymnaea natalensis* Krauss, *Bulinus trigonus* (Martens), *Bulinus forskalii* (Ehrenberg), *Biomphalaria sudanica* (Martens), and *Anisus natalensis* (Krauss) were abundant in the shallow water with rich vegetation. No snails were found to be naturally infested with the larval stages of *Fasciola*. However, since all the *Fasciola* species of which the snail hosts are known develop in species belonging to the genus *Lymnaea sensu lato*, it was suggested that *L. natalensis* might be a snail host of *Fasciola nyanzae*. Accordingly laboratory-bred specimens of this snail were exposed to miracidia when they had hatched from the eggs in the laboratory.

The miracidium of *F. nyanzae* is 163 to 212 μ long and is identical with the miracidium of *F. gigantica* in structure. It is covered with twenty-one ciliated epithelial cells arranged in five transverse rows of 6, 6, 3, 4 and 2 cells respectively.

In three experimental series 98 young specimens of *L. natalensis* were exposed separately to 4 to 6 miracidia each. After this exposure the snails were placed in aquaria, each series being kept separately, in a room at a temperature of 26°C. By dissecting these snails we were able to follow the larval development of *F. nyanzae* from sporocysts to cercariae. In addition a number of young snails were exposed to miracidia by placing them all together in water with numerous swimming miracidia. These snails were kept until the emergence of cercariae which, after encystment, were used in an attempt to infect calves, goats and rabbits with *F. nyanzae*.

The first dissection was made 6 days after exposure of the snail to miracidia and 3 sporocysts were recovered. The elongated bodies of these sporocysts measured 0·38 mm., 0·40 mm. and 0·41 mm. in length respectively. The remains of the eye spots of the miracidia, in the form of two granular groups of pigment floating in the body wall, were clearly seen in all these sporocysts. A single redia had developed in the body cavity of each sporocyst by that time. These rediae were 0·31 mm., 0·27 mm. and 0·22 mm. long respectively and each had a well developed pharynx and the gut was easily seen. In one sporocyst, in addition to the young redia, a small embryo ball was seen lying at the end of the body cavity (Fig. 3A), while in two other sporocysts only a few cells, presumably germinal cells, were observed attached to the wall at the end of the body cavity.

Young rediae of the first generation measuring 0·30 to 0·34 mm. in length and lying free in the tissue were recovered from a snail dissected on the 7th day after exposure to miracidia. Each redia possessed a strongly developed collar ring and a pair of locomotor appendages. In the body cavities of these rediae germinal cells and a few embryo balls were present (Fig. 3B).

The next dissection was made 19 days after the exposure, when a snail was found harbouring six rediae with daughter rediae inside their body cavities. These mother rediae measured from 1·15 mm. to 1·42 mm. in length and contained many embryo balls and from 2 to 5 daughter rediae (Fig. 3C). Also present in the snail were 14 very young rediae with slender bodies, 0·35 to 0·85 mm.

3

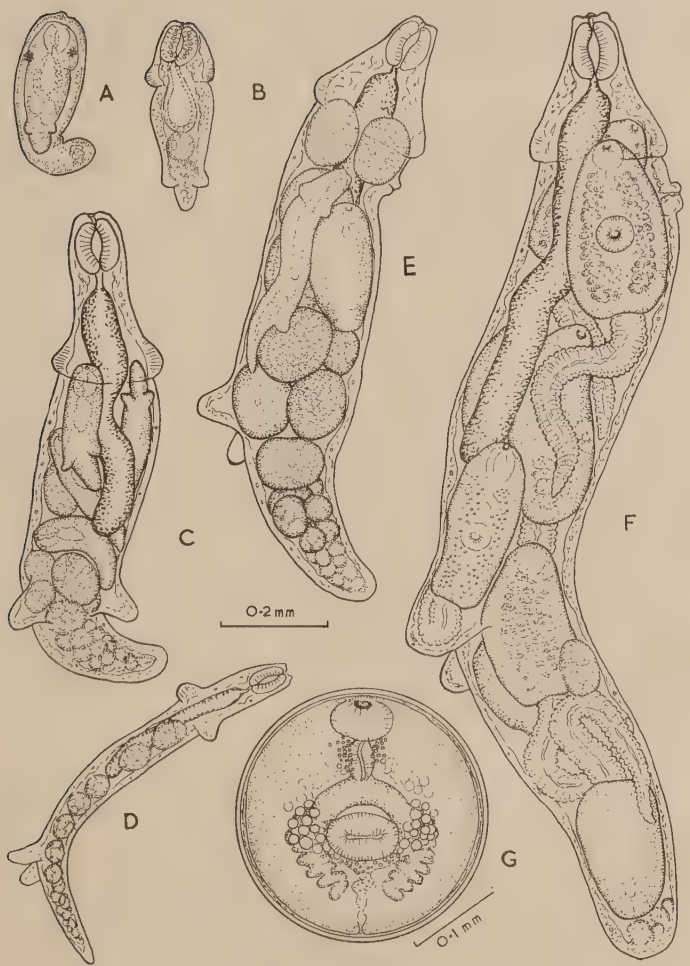


Fig. 3.—Larval stages of *Fasciola nyanzae*. **A**=sporocyst; **B**=newly born redia of the 1st generation; **C**=1st generation redia in the phase of redial production; **D**=newly born redia of the 2nd generation; **E**=1st generation redia with a daughter redia and cercarial embryos; **F**=1st generation redia with developing cercariae; **G**=metacercaria.

long, containing embryo balls lying one after another in a line in the body cavity (Fig. 3D) ; all these rediae were found in the liver of the snail and all are, apparently, rediae of the second generation which had left their mother rediae recently.

In snails dissected on the 28th day of the larval development many rediae of the first generation were seen, containing from 1 to 6 daughter rediae and developing cercariae as well as many embryo balls (Fig. 3E). Second generation rediae, which became more numerous in the snails than rediae of the first generation by that time, varied from newly born specimens, which were very tiny and contained a few small embryo balls, to rediae 1·0 mm. long, each containing up to ten large embryo balls in the body cavity.

On the 40th day after exposure to miracidia, the first generation rediae were found to contain maturing and developing cercariae and a few embryo balls. No daughter rediae or redial embryos were seen in these rediae, whose bodies varied from 1·41 mm. to 2·24 mm. in length. The total number of embryos and cercariae seen in the body cavity of a redia at any one time varied from 7 to 20 (Fig. 3F). Second generation rediae found in the snails dissected on the 40th day measured from 1·0 mm. to 1·40 mm. in length and contained either daughter rediae (third generation) and embryo balls or daughter rediae, cercarial embryos and embryos balls. A few newly born young rediae were recovered from the snails. The rediae of the first and second generation were found to be similar to those of *F. gigantica* except that the collar ring in rediae of *F. nyanzae* looks more conspicuous and muscular than it does in the former species.

The first emergence of cercariae from the snails kept at 26°C. was observed 44 days after their exposure to miracidia and from then on they emitted small numbers of cercariae daily. After swimming in the water for a short time the cercariae attached themselves to the wall of the aquarium or, preferably, to vegetation near the water surface and encysted. The cysts of *F. nyanzae* are white or creamy-white in colour like the cysts of *F. gigantica* but they are larger and more flattened. The outer diameter of complete cysts of *F. nyanzae* varies from 242 μ to 272 μ ; the cysts without the outer fibrous layer are lens-shaped in appearance and measure from 212 μ to 228 μ in diameter and about 45 μ in thickness. The metacercaria of *F. nyanzae* is similar, except that it is larger in size than that of *F. gigantica* (Fig. 3G).

Only two infected snails were dissected after they had begun to emit cercariae. One snail was dissected 70 days after its exposure to miracidia and numerous rediae were recovered from it, ranging from large rediae, 2·6 mm. to 3·4 mm. long, containing up to 21 developing and maturing cercariae and embryo balls, to newly-born rediae, 0·6 mm. long, with only a few small embryo balls.

The second snail, the last of the series of infected specimens, was dissected on the 122nd day, when 365 rediae were recovered from it. Of these only five were small, young rediae, the rest were mature and contained developing and maturing cercariae and embryo balls. No daughter rediae or redial embryos were seen in these parent rediae.

In an attempt to infect domestic animals experimentally, encysted cercariae of *F. nyanzae* were fed to the following animals : three calves had 115, 192 and 440 cysts respectively, three goats had 45, 50 and 60 cysts respectively and five rabbits had 15, 15, 20, 20 and 40 cysts respectively. After a period of 90 days the faeces of these animals were examined regularly but no eggs were recovered. The goats and rabbits were sacrificed and dissected 4 months after the metacercariae had been given to them but no liver flukes were recovered and no lesions were seen in the livers of these animals. The calves have not shown any signs of illness since they were fed with the cysts, have passed no liver fluke eggs in their faeces, and, although they were not sacrificed and dissected, it became evident that *F. nyanzae* had not been able to infect them. It is worth mentioning that calves and goats are very susceptible to infection with *F. gigantica* and about 50 to 70 per cent. of the metacercariae given to these animals grow into mature flukes in their livers. It seems that *F. nyanzae* is a specific parasite of the hippopotamus which normally cannot parasitize other animals.

Discussion

Fasciola nyanzae Leiper is more closely related morphologically to *Fasciola hepatica* L. and *F. gigantica* Cobbold than to *Fascioloides magna* (Bassi), with which Jackson (1921) has grouped the liver fluke of the hippopotamus. In all the specimens of *F. nyanzae* which we have examined the yolk glands lie on both ventral and dorsal sides of the gut ramifications, as in the other two species belonging to the genus *Fasciola*. From these two species *F. nyanzae* is clearly distinguished by the fact that the area occupied in the

body by the ramified testes is smaller. Absence of ramose internal diverticula of the gut in *F. nyanzae* also distinguishes this species from *F. gigantica* which, however, it resembles in size and to some extent in the shape of the body.

The observations on the larval development of *F. nyanzae* have shown that it differs from that of *Fascioloides magna* and is similar to the larval development of *F. gigantica*. The sporocyst of *F. nyanzae* is identical to that of *F. gigantica* although it gives birth to a smaller number of rediae, probably no more than three, than does the sporocyst of *F. gigantica*, which produces up to six rediae in the same snail host. The rediae of *F. nyanzae* are also similar to those of *F. gigantica*, except that the collar ring in *F. nyanzae* rediae is more conspicuous. They produce daughter rediae and cercariae like those of *F. gigantica* (Dinnik and Dinnik, 1956) and unlike those of *Fascioloides magna*, which are much larger and contain about a hundred cercariae each (Sinitsin, 1934). All these facts have convinced us that the liver fluke from the hippopotamus should remain in the genus *Fasciola* L., to which the two well-known species, *F. hepatica* L., *and F. gigantica* Cobbold, belong.

Acknowledgments

The writers wish to thank Mr. R. Bere, Director of the Uganda National Parks, and members of his staff for their help and the interest which they have taken in this work. They are also extremely grateful to Dr. W. M. Longhurst, who made the investigation possible, and to Mr. T. J. Coyle for the specimens of *F. nyanzae* which he collected.

References

Dinnik, J. A. and Dinnik, N. N., 1956.—"Observations on the succession of redial generations of *Fasciola gigantica* Cobbold in a snail host." *Z. Tropenmed. u. Parasit.*, **7**, 397—419 (W.L. 23593a).

Jackson, H. G., 1921.—"A revision of the genus *Fasciola*. With particular reference to *F. gigantica* (Cobbold) and *F. nyanzi* (Leiper), [*F. nyanzae* Leiper]." *Parasitology*, **13**, 48-56 (W.L. 16035).

Leiper, R. T., 1910.—"The entozoa of the hippopotamus." *Proc. zool. Soc. Lond.*, 233–251 (W.L. 16969b).

Sinitsin, D. F., 1934.—"Studien über die Phylogenie der Trematoden. VI. The life histories of some American liver flukes." *Z. Parasitenk.*, **6**, 170–191 (W.L. 23536b).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 63–76.

Estimation of Field Populations of Cyst forming Nematodes of the Genus *Heterodera*

By D. W. Fenwick*
Red Ring Research Scheme, Trinidad

Despite many recent advances in the study of nematicides and in the breeding of resistant strains of plants, control of cyst forming nematodes is still best accomplished by crop rotation ; to be fully effective, it is also desirable that a field in which it is proposed to grow a susceptible crop should be sampled and an estimate made of the population of nematodes present : such an estimate is susceptible to different sources of error and although it is possible to decrease the magnitude of these errors a compromise has to be reached between the conflicting claims of precision on the one hand and the expenditure of man-power on the other ; it is desirable that this compromise be based on factual data rather than guesswork ; good estimates of the order of magnitude of the different types of error are therefore desirable.

It is convenient to classify the errors which influence the accuracy of a final estimate into those occurring in the field and those occurring in the laboratory. The errors inherent in laboratory processing of soil samples have already been discussed (Fenwick, 1959) and it is proposed in this paper to discuss errors incurred as a result of different sampling procedures adopted in the field.

Various criteria have been adopted by different workers—the number of cysts per mass of soil, the number of "full cysts", the number of "viable" cysts, as well as the number of larvae available per mass of soil. Present discussion is confined to the first and the last of these criteria.

Most workers when sampling a field, consider it to be made up of one or more areas and take a fixed number of samples from each of these areas ; the samples from each area are mixed to form a single bulk sample which is considered to be representative of the sub-area. The validity of such a technique was examined by Jones (1945) who took two sets of forty-eight samples from a two acre plot,

* Previously at Rothamsted Experimental Station.

examined each sample separately and recorded the number of cysts, full cysts and total available larvae per sample. He pointed out that in an ideal bulk sample, each individual sample or boring should be of equal weight ; his individual samples did not conform with this criterion since their average weight was $102{\cdot}5 \pm 0{\cdot}15$ gms. for his first set of forty-eight samples and $101{\cdot}7 \pm 2{\cdot}45$ for his second ; this suggests that the weight of his individual samples was subject to a standard error of approximately 16 gms. Despite this, analysis of his data showed that results for "ideal" bulk samples were almost identical with those obtained from his own bulk samples made up with no regard to the weights of individual corings. Results from the two sets of samples did not differ significantly from one another and he therefore concluded that the pooling of individual samples into a bulk sample was a justifiable procedure. Analysis of the data, using cyst count as a criterion, showed that the standard error expressed as a percentage of the mean varied from 10% for the mean of ten borings to 3% for the mean of fifty borings : he concluded that an increase in accuracy beyond this level could only be obtained by a great increase in the number of borings and was not usually justified.

In 1946 the Conference of Advisory Entomologists appointed a sub-committee to consider the problem of soil sampling ; it was recommended that fifty samples should be taken for an area of up to ten acres and that larger fields should be sub-divided into ten acre plots ; the fifty samples should be taken in a random manner over the whole field and methods were suggested to accomplish this : the fifty samples should be pooled and passed through a 2 mm. sieve prior to mixing and taking a 100 gm. sub-sample for examination.

Anscombe (1950) discussed the statistical implications of these recommendations : after examining data assembled by different workers, he decided that counts from a series of borings taken at random over an area follow the negative binomial distribution and derived a measure k to express the uniformity of an infestation. He concluded that if 25 borings were taken and pooled, it would be reasonable to expect that the percentage standard error when a 50 gm. sub-sample was examined, would be approximately 20% ; no great advantage would accrue from increasing the number of sampling points without also increasing the size of the sub-sample.

Fenwick (1959) discussing errors due to the uneven distribution of cysts in a field, pointed out that if it were desired to sample a given field to a predetermined level of accuracy, it was first necessary to take a preliminary set of samples to estimate the heterogeneity and to

use this in preparing a suitable sampling plan. He also referred to an investigation undertaken at Rothamsted on land which had grown potatoes annually for six years and on which the infestation should therefore be relatively uniform ; he suggested that when a composite sample made up of 400 cores was taken from this area, the overall percentage error would vary from 55% when four cysts were counted to 9% if 400 cysts were counted. The data referred to are a part of those presented in this paper.

Fidler, Church and Southey (1959) working with *H. major* Schmidt, showed that samples taken from points one yard apart varied less than if they were more widely distributed ; for a given level of infestation of 1·0 cysts per gm. and a given weight of soil examined, the accuracy is greatest when the bulk sample is made up of approximately equal weights of soil taken from a large number of randomly located points but there is a limit to the accuracy attainable for any given sample weight, however many cores are taken. Thus if only 500 gms. of soil are examined, there is no point in taking more than 25 corings when an accuracy of 10% is to be expected ; if 2,500 gms. of soil be examined from 50–100 sampling points, an accuracy of 4% is likely to be attained.

In the present paper data are recorded as a result of grid sampling a number of fields on the Rothamsted farm which show different levels of infestation. Information is presented on the magnitude of the errors likely to be encountered when bulk samples of different composition are examined.

Technique

Duplicate samples were taken from each of a number of points in a square grid pattern. After mixing, a 100 gm. sub-sample was examined from each individual sample. Cysts were recovered using the Fenwick can and slide (Fenwick, 1940). Larval counts were estimated by withdrawing batches of cysts using the Hesling aspirator (Hesling, 1952) and dissected using the Reid crushing slide (Reid, 1955). The liberated eggs and larvae were estimated by making up the suspension to a known volume and counting the number present in a 1 ml. aliquot. It was thus possible to calculate the number of cysts per gm., available larvae per cyst and available larvae per gm. of soil.

The data obtained from these observations were coded to the form $y=100\ (\log x+1)$ where x was the original count obtained by

examining individual samples ; the transformed data were submitted to analysis of variance, the variance between replicate counts being expressed as "error" (V_E) and that between sampling points being expressed as such (V_s).

3,642 soil samples were taken from a total of eleven fields. Zero counts were omitted from the analysis as the meaning of a variance or standard deviation derived from data containing many zeros is doubtful : field averages were calculated from non-zero readings and multiplied up.

Results

A summarized analysis of variance for the eleven fields is presented in Table 1. The "between spot" variance is in most cases greater than the error or "within spot" although not invariably so. Variances for cyst counts are also generally lower than those for larval counts. Variance levels also differ widely from field to field.

If one assumes that heterogeneity is greater as the distance between sampling points increases, it is to be expected that the "within spot" variance should be as low as is consistent with the Poisson distribution. Accordingly the expected Poisson error was estimated on the basis of the mean spot counts, also the actual "within spot" error on the basis of the error variances in Table 1. The results of this are summarized in Table 2. Over the whole series, the actual error is greater than the Poisson, the ratio of actual/Poisson error being 1·77 for cyst counts and 2·56 for larval counts. In the case of cyst counts the actual error is substantially greater than Poisson in four cases (plots 1, 2, 6 and 11) and in no case is it substantially smaller. With larval counts, the actual error is substantially greater in four cases (plots 1, 2, 3 and 4) while in three cases the reverse applies (plots 7, 8 and 10). It is therefore likely that the actual distribution of worms even over extremely small areas is not random.

Referring to Table 1, it can be shown mathematically that the V_s terms for both cysts and for larvae are made up of two additive factors, σ_s^2 the true variance existing between different points on the grids when the density at each point is absolutely determined, plus a factor contributed by V_E: in the case under discussion the true value of σ_s^2 can be shown to be equal to $\frac{V_s - V_e}{20{,}000}$ and it is thus possible to derive a set of values for σ_s^2 each of which is an estimate of field heterogeneity based on the counting of an infinite number of cysts.

Table 1

Analysis of Variance on sampling data (coded as $y = 100 \log (x+1)$) from each of eleven fields examined at Rothamsted.

Plot Field No.	cysts/100 gm. x	cysts/100 gm. V_e	cysts/100 gm. V_s	larvae/gm. x	larvae/gm. V_e	larvae/gm. V_s	No. of non zero observations
1	211·4	129·8	3448·2	202·6	378·5	5162·0	364
2	216·9	96·9	4104·2	204·8	281·1	6792·6	360
3	80·0	166·9	1104·9	33·6	1515·2	3694·5	122
4	129·2	128·3	8617·0	112·4	682·4	15257·6	182
5	56·2	209·8	445·1	4·4	452·0	594·6	189
6	97·8	125·4	907·5	24·4	264·0	4661·4	55
7	95·4	159·5	28287·2	6·5	139·4	411·6	55
8	75·6	185·0	283·2	3·8	94·5	137·0	55
9	101·3	183·8	4927·7	68·1	463·3	11258·4	55
10	99·9	130·6	328·5	4·3	145·4	161·2	55
11	190·2	56·7	2000·0	166·0	224·3	3076·5	55
All fields	151·5	137·25	3221·9	121	456·7	5632·5	1556

TABLE 2

A comparison of "within spot" error with the theoretic Poisson error calculated on the mean count for the eleven fields examined

Plot No.	Cysts $\bar{x}$	Cysts Actual error %	Cysts Poisson error %	Cysts Actual / Poisson	Larvae $\bar{x}$	Larvae Actual error %	Larvae Poisson error %	Larvae Actual / Poisson
1	211·4	30	8·4	3·57	202·6	56	10	5·60
2	216·9	21	8·3	2·53	204·8	46	10	4·60
3	80·0	34	38	0·89	33·6	145	68	2·13
4	129·2	30	27	1·11	112·4	82	25·6	3·20
5	56·2	40	52	0·77	4·4	63	95	0·66
6	97·8	29	10	2·90	24·4	45	71·5	0·63
7	95·4	34	33	1·03	6·5	31	68	0·45
8	75·6	37	41·7	0·88	3·8	25	100	0·25
9	101·3	37	31	0·83	68·1	64	45	1·42
10	99·9	30	33	0·90	4·3	32	100	0·32
11	190·2	73	11·2	6·51	166·0	41	14·6	2·80
General	151·5	31	17·5	1·77	121	64	25	2·56

TABLE 3

Showing the variance of bulk samples taken from a varying number of points when an infinite number of cysts are counted

Plot No.	Cyst Counts No. of cores per sample 1	4	25	100	400	Larval Counts No. of cores per sample 1	4	25	100	400
1	·1659	·0415	·0062	·0017	·0004	·2400	·0600	·0100	·0025	·0005
2	·2003	·1001	·0401	·0020	·0005	·3260	·081	·012	·003	·001
3	·0469	·0117	·0019	·0005	·0001	·1089	·0545	·0044	·0011	·0003
4	·4240	·1060	·0168	·0042	·0010	·7780	·1945	·0312	·0078	·0019
5	·0117	·0029	·0004	·0001	·0	·0071	·0020	·0004	·0001	·0
6	·0390	·0097	·0016	·0004	·0001	·2198	·0549	·2088	·0022	·0005
7	·0064	·0016	·0004	·0001	·0	·1361	·0340	·0056	·0014	·0004
8	·0049	·0012	·0004	·0001	·0	·0043	·0010	·0003	·0001	·0
9	·2372	·0593	·0096	·0024	·0006	·5390	·1350	·0216	·0054	·0014
10	·0198	·0049	·0008	·0002	·0001	·0008	·0002	·0	·0	·0
11	·0716	·0179	·0028	·0007	·0002	·1426	·0356	·0056	·0014	·0004
General	·1540	·0380	·0060	·0015	·0004	·2588	·0647	·0104	·0026	·0006

A bulk sample made up from one core would be subject to this variance; if the bulk samples were made of a number of cores (n) its variance would be $\frac{\sigma^2}{n}$. A set of values for the variance of bulk samples composed of different numbers of cores are set out in Table 3, and these represent the minimum variance to which samples taken from the several fields are subject when data are based on an infinite number of individuals.

TABLE 4

Showing the percentage error to which bulk samples made up of different numbers of cores are subject when each core is made of an infinite quantity of soil and an infinite number of worms are counted

	Cyst Counts No. of cores per sample					Larval Counts No. of cores per sample				
Plot No.	1	4	25	100	400	1	4	25	100	400
1	153	60	20	10	5	209	74	26	11	5
2	180	107	58	11	5	271	91	28	14	2
3	65	28	11	6	2	109	69	16	8	6
4	337	111	35	12	8	662	170	50	22	10
5	28	11	5	2	<1	21	11	5	2	<1
6	57	25	10	5	2	194	71	24	12	5
7	20	9	5	2	<1	134	53	19	9	5
8	18	8	5	2	<1	16	8	4	2	<1
9	207	75	25	12	6	440	133	40	18	9
10	38	17	7	3	2	7	3	<1	<1	<1
11	85	36	13	6	3	139	54	19	9	5
General	147	57	19	7	5	223	79	26	13	6

These figures are in logarithmic form. The square root of these values give the standard deviation in logarithmic form. The antilogs of these latter expressed as 100 (antilog $y-1$) give the error expressed as a percentage of the mean. Such a set of values is presented in Table 4, for the various number of cores taken. These figures represent the minimum error to which an estimate of field density is subject when an infinite quantity of soil is taken from each sampling point and when an infinite number of individual cysts or larvae are counted.

In practice it is only possible to take a limited quantity of soil from each plot and to count a limited number of cysts or larvae. This increases the variance to which data from a bulk sample is subject. If the "within spot" error was of the same order as the Poisson the latter could be used as a basis for adjustment but this is not true for

all the plots and it becomes necessary to multiply the Poisson error appropriate to any given count by the ratio of actual/Poisson error for the plot in question. The standard deviation of an array of logarithmically transformed data when converted back to arithmetic form resolves itself into a multiplication and division factor applicable to the geometric mean of the unconverted data ; thus, if x be an individual value in an array of data and y ($=\log x$) the value when transformed, then an individual y value will be subject to an error of σ_y i.e. $y \pm \sigma_y$; if the antilog of this expression be taken it resolves itself to the form antilog y multiplied or divided by antilog σ. The excess of antilog σ over 1·00 therefore expresses the error as a proportion of the geometric mean. If therefore a mean is subject to a Poisson error, it is possible by adding 1·00 to this error, taking the logarithm of this value and squaring it, to express the variance in a similar form to that in which the "between spot" variance is expressed in Table 3. Using this method the "within spot" variances for different numbers of cysts counted are set out in the first line of Table 5. In the first column of the first section of the table are set out the "between spot" variance for cyst contents of plot 1 for bulk samples made up of 1, 4, 25, 100 and 400 corings. By simple addition of appropriate "within spot" and "between spot" variances it is possible to derive variances appropriate to samples made up of different numbers of borings and different numbers of worms counted. These data comprise the body of this section of the Table. The square root of these values in the second section are a measure of the standard deviation appropriate to such samples. The antilog of these values in the third section give the multiplier/divisor factors while the values decreased by unity and multiplied by 100, presented in the last part of the table give the percentage error to which estimates based on such samples are subject.

By applying this method, errors have been calculated for cyst and for larval counts for each of the eleven fields and are presented in Figs. 1 and 2. A similar set of errors has been calculated for all eleven fields grouped together and is presented in Figs. 3 and 4. Referring to Figs. 1 and 2, it will be seen that the curves for individual plots vary considerably from field to field : thus for cyst counts, the curves for plots 1, 2, 4 and 9 are relatively widely separated while in the case of plots 3, 5, 7 and 10 they are so close together that it has been impossible to present curves based on the higher number of corings which have therefore been omitted : this is also the case for larval counts from plots 5, 6, 7, 8 and 10. In some cases also the curves are very steep while in others they are comparatively level.

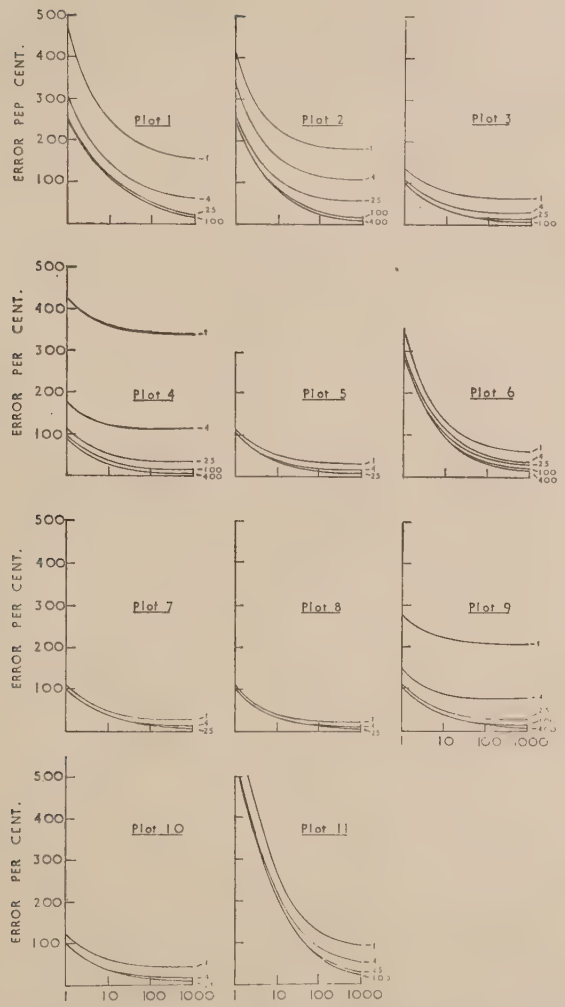


Fig. 1.—Showing the percentage errors applicable to cyst counts from each of eleven plots when the estimates are based on samples composed of different numbers of cores and different numbers of cysts counted. Numbers of cysts counted is plotted horizontally.

The actual form of the curve depends on the relative values of the original estimates of σ_s^2 on the one hand and of the ratio of actual/Poisson variance for "within spot" samples. If σ_s^2 is high—i.e. the plot is very heterogeneous but the "within spot" variance is low (as in plot 4, cyst counts), the slope of each curve is slight since "within spot" variability contributes little to the overall variance which latter is governed primarily by the number of cores taken, so the

TABLE 5

Showing the deviation of percentage error applicable to soil samples based on varying numbers of cores and varying numbers of worms counted

No. of Cores	σ_s^2 (log)	σ_e^2 for different numbers counted				
		1	4	25	100	400
		0·44	0·20	0·06	0·02	0·005
1	0·1659	0·6059	0·3659	0·2259	0·1859	0·1709
4	0·0415	0·4815	0·2415	0·1015	0·0615	0·0465
25	0·0062	0·4462	0·2062	0·0662	0·062	0·0112
100	0·0017	0·4417	0·2017	0·0617	0·0217	0·0067
400	0·0004	0·4404	0·2004	0·0604	0·0204	0·0054
		σ_s (log) for different numbers counted				
1	0·407	0·778	0·605	0·475	0·4314	0·4134
4	0·204	0·693	0·481	0·3186	0·2479	0·2156
25	0·0787	0·668	0·454	0·573	0·1619	0·1055
100	0·0412	0·665	0·449	0·2483	0·1473	0·0818
400	0·200	0·664	0·448	0·2457	0·1428	0·0734
		σ_s (arithmetic) for different numbers counted				
1	2·53	6·00	4·02	2·990	2·690	2·590
4	1·60	4·93	3·02	2·08	1·77	1·64
25	1·19	4·66	2·84	1·81	1·45	1·27
100	1·10	4·62	2·81	1·77	1·40	1·21
400	1·05	4·61	2·80	1·76	1·93	1·18
		% error for different numbers counted				
1	153	500	302	199	169	159
4	60	393	202	108	77	64
25	19	366	184	81	45	27
100	10	362	181	77	40	21
400	05	361	180	76	39	18

curves are widely spaced : it is only when 25 or more corings are taken that the number of cysts counted have a great influence on the final error. If field heterogeneity is of a low order (plot 6, cyst counts) and "within spot" variability is high then the curves are steep since the number of cysts counted has a big effect on accuracy ; under such conditions, increasing the number of cores taken has little effect and the curves for different numbers of cores are closely spaced. If

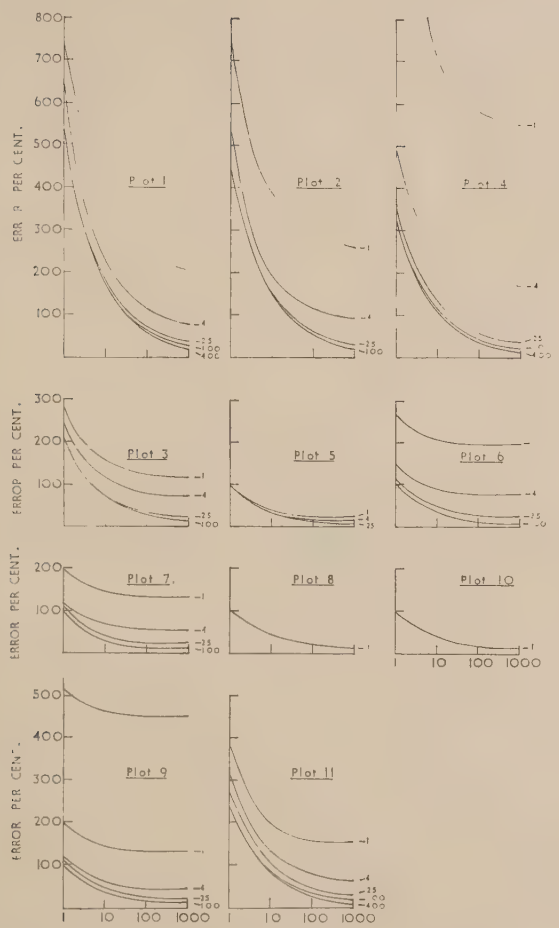


Fig. 2.—Showing the percentage errors applicable to larval counts from each of eleven plots when the estimates are based on samples composed of different numbers of cores and different numbers of larvae counted. Number of larvae counted is plotted horizontally.

field heterogeneity and "within spot" variability are both high (plot 1, cyst counts) then initial errors based on one core and one worm counted are very high but the curves are steep and widely separated since both core number and number of cysts counted have comparable effects on overall error. If field heterogeneity and "within spot" variability are both small (plot 7 cyst counts) then the initial error based on one core and one worm counted will be low and the curves not steep and also closely spaced.

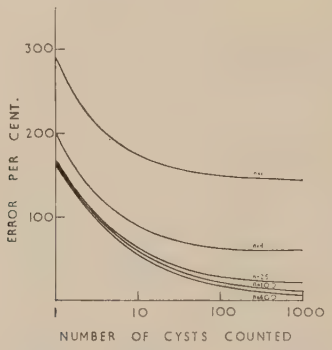


Fig. 3.—Showing the general percentage error applicable to cyst counts for eleven fields pooled, when the estimates are based on samples containing different numbers of cores and different numbers of cysts are counted.

Table 6

Showing likely error % for samples composed of twenty-five corings when 100 individuals are counted, for the eleven fields under study

Plot No.	1	2	3	4	5	6	7	8	9	10	11	General
Cyst counts	45	67	48	37	11	32	11	11	28	12	68	27
Larval counts	65	57	28	64	11	26	22	11	42	9	44	39

It will thus be seen that there is considerable variation in the errors to which a given sampling technique is subject. To illustrate the magnitude of these differences, data are given in Table 6 for errors in cyst and in larval counts for each of the eleven plots when the bulk sample is based on 25 borings and 100 worms are counted : it will be seen that errors in cyst count fluctuate from 11–68% and larval counts from 11 to 65%. It will be seen that in general larval counts are more variable than cyst counts (39% against 27%). It is therefore quite impossible to forecast the magnitude of the error to

which a given sample, based on a given number of cores and given number of worms counted, will be subject and further emphasis is therefore lent to the writer's original suggestion that if it is necessary to sample a field to a predetermined level of accuracy, preliminary samples must be taken to obtain information on field heterogeneity and other factors.

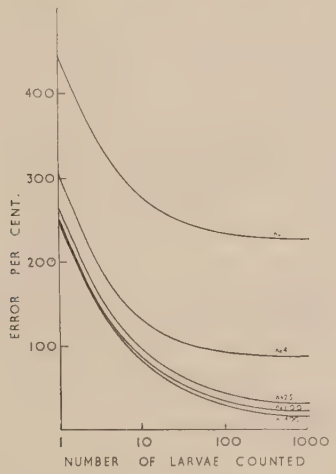


Fig. 4.—Showing the general percentage error applicable to larval counts for eleven fields pooled, when the estimates are based on samples containing different numbers of cores and different numbers of larvae are counted.

In view of the lack of constancy between different fields it is considered that a great deal of importance should not be attached to Figs. 3 and 4; not only is it impossible to suggest that these curves represent general levels of error likely to be encountered in fields generally, they do not even represent the true state of affairs in many of the fields under discussion. Their importance is that they emphasize the magnitude of the errors to which a soil sampling technique is subject, but these errors may differ widely in practice from the estimates given by these curves.

Despite the variability in the form of the curves presented they all show one feature in common—the increase in accuracy by taking more than twenty-five borings when 100 worms are counted is very small and not in proportion to the increase in labour involved: even if 1,000 worms be counted the same applies to a less extent. The difference in accuracy from taking 100 borings as against 400 is so

small as to be scarcely perceptible in Figs. 1 and 2. It is therefore reasonable to suppose that if 25–100 borings be taken from a plot, even though the actual precision of the final estimate will be variable, yet a reasonable balance will have been achieved between accuracy and the expenditure of time.

Conclusions

Fields differ markedly from one another both in density of infection with cyst forming nematodes and also in the uniformity of distribution.

In most cases composite samples taken from such fields should be made up of 25–100 borings—the increase in precision by taking more than this number is rarely justified.

The accuracy of an estimation based on samples taken in this manner is variable and it is not possible to forecast it beforehand.

If it is desired to sample a field to a predetermined level of accuracy preliminary samples should be taken in order that estimates can be made of heterogeneity. The final sampling procedure should then be based on these preliminary estimates.

Acknowledgments

The writer is indebted to Mr. M. H. Westmacott, for considerable help and advice on the statistical aspects of this work.

He also wishes to thank Mrs. E. Widdowson who supervised the greater part of the routine work and the statistical analysis of the data. His thanks are also due to Mrs. M. Mohammed who was responsible for setting out and drawing the graphs herein presented.

References

Anscombe, F. J., 1950.—"Soil Sampling for Potato Root Eelworm cysts." *Ann. appl. Biol.*, **37**, 286–295. (W.L. 1025.)

Fenwick, D. W., 1940.—"Methods for the Recovery and Counting of cysts of *Heterodera schachtii* from soil." *J. Helminth.*, **18**, 155–172. (W.L. 11224c.)

————, 1959.—"Estimation of Eelworm Populations : Principles and Techniques—Ministry of Agriculture, Fisheries & Food." *Plant Nematology Technical Bulletin No.* 7, 115–118.

Fidler, J. H., Church, B. M. and Southey, J. F., 1959.—"Field sampling and laboratory examination of cereal root eelworm cysts." *Plant Path.*, **8**, 27–34.

Hesling, J. J., 1952.—"An improved method of separating eelworm cysts from debris." *J. Helminth.*, **26**, 69–70. (W.L. 11224c.)

Jones, F. G. W., 1945.—"Soil Populations of Beet Eelworm (*Heterodera schachtii* Schm.) in Relation to Cropping." *Ann. appl. Biol.*, **32**, 351–380. (W.L. 1025.)

Reid, E., 1955.—"A Rolling method for opening cysts of Potato Root Eelworm." *Plant Path.*, **4**, 28–29.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 77–84.

Records of Mortality from Helminth Diseases in England and Wales up to the Year 1880

By A. E. Fountain

Formerly Deputy Director, Commonwealth Bureau of Helminthology

Material for the period covered by this paper is very meagre and in the published figures from which we can draw there is very little detail of specific infections. The only significant sources are the London Bills of Mortality and the Annual Reports of the Registrar-General.

The Bills of Mortality

Although the recording of burials by parish priests had been mandatory in England since 1538 it was not until bills of mortality began to be issued regularly at the beginning of the 17th century that collected records were published. Bills of mortality were not national in scope but were prepared for various towns up and down the country. Only the London series, however, is well known and is sufficiently continuous to allow of detailed study; it is the only series dealt with here.

Although there were some earlier issues, the London Bills did not appear regularly until 1603. They continued without a break until the mid-nineteenth century and were published every week, with an annual summary. From 1629 causes of death, grouped under about 60 heads, were included. This account of how causes of death were ascertained, and of how the Bills were drawn up, comes from John Graunt (1676) :—

"The searchers hereupon (who are ancient Matrons, sworn to their office) repair to the place where the dead Corps lies, and by virtue of the same, and by other enquiries, they examine by what Disease or Casualty the Corps died. Hereupon they make their Report to the Parish Clerk, and he, every Tuesday night, carries in an Accompt of all the Burials and Christenings happening that week, to the Clerk of the Hall. On Wednesday the general Accompt is made up and printed, and on Thursday published and dispersed to the several Families who will pay four Shillings per Annum for them."

The nomenclature of disease used for the Bills remains more or less the same, at least until the 18th century, and includes such items as "Colick, griping and twisting of the guts", "Rising of the lights", "Stagnation in the head" and "Surfeit". The heading "Worms" is used throughout although in some early years there is a combined head for "Teeth and worms". We have no information, of course, as to how the "ancient matrons" diagnosed "worms" and it is even possible that the term as used here has no helminthic connotation. But for what they are worth Table I gives, from the annual summaries, the figures for "worms" for the years 1630 to 1755, together with total burials. To save space only every fifth year is included.

Table I

Deaths ascribed to "worms" in the London Bills of Mortality

Year	Burials	Died of "Worms"	Year	Burials	Died of "Worms"
1630	9237	31	1695	19047	76
1635	10651	27	1700	19443	53
1640	11321	†	1705	22097	31
1645	9608	†	1710	24620	75
1650	8749	65	1715	22232	44
1655	11348	†	1720	25454	75
1660	15118	†	1725	25523	34
1665	97306*	†	1730	26761	15
1670	20198	†	1735	23538	9
1675	17244	44	1740	30811	13
1680	21053	35	1745	21296	13
1685	23222	17	1750	23727	5
1690	21461	29	1755	21917	11

* including 68596 of the plague † not separately recorded

Registrar-General's Reports

The Bills of Mortality had served a useful purpose but the system of parochial registration was not really satisfactory and a Select Committee of the House of Commons was set up in 1833 to look into the whole question. This Committee's report led to the Births, Marriages and Deaths Registration Act of 1836 which provided for the setting up of a General Register Office and the appointing of a Registrar-General. The Act came into operation in July 1837 and for the first time a national system for recording causes of death in England and Wales came into force. It is from the annual reports of the Registrar-General that figures for mortality from helminth diseases must now be sought.

Nomenclature and Classification of Disease

From the first the General Register Office had the benefit of the services of William Farr, the pioneer medical statistician, who was appointed "Abstractor of Statistics" and who remained in office until 1880. It was Farr who drew up the classification of disease used in the annual reports.

The initial system, which was used for the first to twentieth reports (1837–1857), consisted of two main classes : (i) Fatal Diseases and (ii) Sporadic Diseases. Under the first were listed all epidemic diseases, while Sporadic Diseases were divided into the following groups :—

Diseases of the nervous system and senses
Diseases of the organs of respiration
Diseases of the organs of circulation
Diseases of the digestive organs
Diseases of the urinary organs
Diseases of the organs of locomotion
Diseases of the integumentary system
Diseases of uncertain seat.

Worms were included under "Diseases of the digestive organs" under a subhead "Intestinal canal" and a note states that "Worms" includes "Tape Worm (Taenia), Worm Fever, Infantile Remittent Fever".

In the fourth report of the Registrar-General, for 1840, Farr includes (p.147) notes for the use of those who return causes of death. Under "Worms" the following subheads, with synonyms, are given :—

Head or Subhead	*"Synonyms which may be used at the discretion of the medical informant if considered more appropriate in the particular case"*
Worms	Vermes: verminatio
Hydatids	Acephalocystis endogena: A. Multi fida: Echinococcus hominis
Tapeworms	Taenia solium
Strongylus gigas	
Ascaris lumbricoides	Round Worm
Ascaris vermicularis	Thread Worm

A note adds :—

"Other parasitic animals, and indeed those above-named, though frequent complications of infantile diseases, rarely prove fatal. When such curious species as the *Trichinella Spiralis* are discovered, they should be mentioned after the fatal disease."

This classification remained in use up to the 20th Report (1857). In spite of the subdivisions of "Worms" listed above, the actual tables, as we shall see, give very little detail and most deaths are simply attributed to "Worms", with a few cases of hydatid or tapeworm specially noted. For the years 1847 to 1855 inclusive the tables do not mention "Worms" at all.

In 1853 Farr attended the International Statistical Congress in Paris and was there commissioned to help in the preparation of an international classification of disease. To this end he revised the system in use at the General Register Office for consideration at the next congress. This revised scheme was set out in the 16th Annual Report, 1853 (Appendix, pp.71–105). It places Parasitic Diseases in Order 4 (Parasitici) of Class 1 ; Zymotic Diseases (Zymotici). Worms (Vermes) are a group of the Parasitic Diseases and there are the following subdivisions :—

(a) Hydatids (Acephalocystis, Echinococcus hominis)
(b) Tape worm (Taenia solium)
(c) Strongilus gigas
(d) Round worm (Ascaris Lumbricoides)
(e) Thread worm (Ascaris Vermicularis)
(f) Guinea worm (Dracunculus)

As a sample of the new scheme, causes of death for females in England in 1852 are classified by it. Under "Worms" the incidence is "nil".

Farr's revised classification was unobtrusively introduced in the 21st Annual Report (1858) and was still in use at end of the period covered by this paper.

William Farr's Comments on the Tables

Most of the annual reports of the Registrar-General include illuminating comments by William Farr on points brought out by the tables which he considers to be worthy of special notice. These comments take the form of a letter to the Registrar-General. A selection from his remarks on helminth infections is given below.

From the 22nd Report, 1859 (p.187) :—

"The ova of worms must be derived generally from impure river waters into which the refuse of towns is poured ; and although they do not frequently prove fatal, they are troublesome and nauseous, and often the cause of disturbance in the system."

From the 23rd Report, 1860 (p.332) :—

"Worms and other parasitic diseases, so rare now and so common in the middle ages, were the cause of 167 deaths."

From the 24th Report, 1861 (p.220) :—

"It is worthy of remark that girls suffer more than boys from worms ; thus the death of 71 girls, and of only 41 boys under 5 years of age, were referred to worms, and in after life an equal proportion prevails. The female tissues perhaps afford the most fruitful nidus for parasites."

From the 25th Report, 1862 (p.185) :—

"Parasitic diseases diminish with the progress of cleanliness ; worms enter the body through water generally, and sometimes through diseased meat : 156, chiefly children, died of worms. Should arrangements be made to dispose of the sewerage of towns, and to supply houses with pure water, we may hope to see the deaths by worms diminish."

From the 32nd Report, 1869 (p.217) :—

"Parasites, including hydatids, itch insects, tape worms, and worms of all descriptions still cause a certain number of deaths, which will probably diminish as the water-supply becomes purer, and the meat-supply is watched . . . The means of personal cleanliness, soap and abundance of pure water, should be placed within the reach of the whole population . . . and some means of personal coercion, if necessary, might well be employed, to prevent unclean vagrants carrying and spreading infection among their race."

From the 33rd Report, 1870 (p.405) :—

"The ova of worms are at times conveyed into the system by water ; and it is always dangerous, therefore, to drink the water of rivers into which watercloset sewers empty. There is no reason to believe that these ova, any more than the zymotic *biads* (formed after the model of *monad* for the elementary organic corpuscle,

sometimes called cell, by Mr. Darwin gemmule), are oxidized; instead of being destroyed they may be sustained by oxygen."

Mortality from Helminth Diseases, 1837–1880

An attempt has been made in Table II to set out in as convenient a way as possible the figures for deaths from helminth infections given in the Registrar-General's annual reports for the years 1837 to 1880 (1st to 43rd reports). The year 1880 has been taken as a convenient finishing point since William Farr retired from the General Register Office in that year and an epoch ends.

In addition to the figures in the table it should be noted that from the year 1858 separate figures are given for London (in which worms were separated from other parasites) and for each of the eleven divisions into which England and Wales was divided (listed under Parasites only). From 1856 figures for causes of death are also presented in 16 age-groups.

In Table II the population figures for England and Wales are included to enable a more accurate picture of mortality to be obtained.

Table II

Mortality from Helminth Infections in England and Wales, 1837–1880

Year	Population	Deaths from Worms M	F	Total	Additional Notes
1837 Jul.–Dec.	?	119	145	264	Tapeworm in a female aged 25; 1 case of hydatid in abdomen and liver originating in an injury, male aged 30
1838	15,312,256	30	419	749	Hydatid in brain, 1 case; worms in brain, 1 case
1839	15,515,296	342	431	773	Hydatid in brain, 2; hydatid in liver, 1; hydatid in pelvis (female aged 34)
1840	15,721,029	339	374	735*	Tapeworm in female aged 57; hydatids: brain (f. aged 31), abdomen (m. 35), womb (f. 27)
1841	15,929,492	311	337	671*	Tapeworm in females, 2 (aged 1 and 42); "Taking medicine for worms", f. aged 24; hydatid of liver f. aged 4
1842	16,123,793	329	380	726*	Hydatid of uterus, f. aged 33
1843	to 1855 no separate figures for worms for England and Wales as a whole.				
1856	19,042,412	112	139	251	Tapeworms, 2 m. 1 f.; hydatids in liver, 5 m. 5 f.
1857	19,256,516	49	66	115	Hydatids in liver, 7 m. 2 f.
1858	19,471,291	61	89	150	Tapeworm, 1 m. 2 f.; hydatids in liver 1 f.; Lumbricales, 1 f.; Ascarides, 1 f.
1859	19,686,701	70	85	155	Tapeworm, 1 f.
1860	19,902,713	86	81	167	Tapeworm, 1 m. 3 f.

1861	20,119,314	56	101	157	Tapeworm, 1 m. 5 f.; Ascaris lumbricoides, 1 m.
1862	20,336,467	72	84	156	Tapeworm, 1 m. 3 f.
1863	20,554,137	83	115	198	Tapeworm, 1 m. 2 f.; hydatid of liver 1 f.
1864	20,772,308	77	88	165	Tapeworm, 5 m. 3 f.; hydatid of liver, 6 m. 6 f.
1865	20,990,946	78	105	183	Tapeworm, 2 m. 3 f.; hydatid of liver, 5 m. 12 f.; Trichinella spiralis, 1 m.
1866	21,210,020	91	75	166	Tapeworm, 1 m. 3 f.; hydatid of liver, 4 m. 3 f.
1867	21,429,508	81	91	172	Tapeworm, 2 m. 4 f.; hydatid of liver, 12 m. 10 f.
1868	21,649,377	81	91	172	Tapeworm, 6 m. 2 f.; hydatids, 12 m. 8 f.
1869	21,869,607	71	77	148	Tapeworms, 1 m. 2 f.; hydatids, 10 m. 10 f.
1870	22,090,163	71	80	151	Tapeworms, 3 m. 3 f.; hydatids, 8 m. 25 f.
1871	22,782,812	72	88	160	Tapeworms, 2 m. 1 f.; hydatids, 15 m. 22 f.
1872	23,067,835	84	70	154	Tapeworms, 2 m. 3 f.; hydatids, 26 m. 15 f.
1873	23,356,414	87	96	183	Tapeworms, 2 m. 1 f.; hydatids, 13 m. 21 f.
1874	23,648,609	96	92	188	Tapeworms, 4 m. 1 f.; hydatids, 13 m. 16 f.
1875	23,796,072	70	88	158	Tapeworms, 2 m. 3 f.; hydatids, 21 m. 22 f.
1876	24,145,938	71	91	162	Tapeworms, 2 f.; hydatids, 13 m. 18 f.
1877	24,700,853	51	101	152	Tapeworms, 6 f.; hydatids, 27 m. 24 f.
1878	25,009,867	81	86	167	Tapeworms, 2 m. 2 f.; hydatids, 28 m. 31 f.
1879	25,273,981	72	89	161	Tapeworms, 4 m. 1 f.; hydatids, 22 m. 29 f.
1880	25,636,000	85	83	168	Tapeworms, 4 m. 6 f.; hydatids, 21 m. 39 f.

* The discrepancy between sex incidence and total incidence is explained by the fact that figures for each sex do not include London while the total does.

For the years 1843 to 1846 there are figures for London and for 1843 figures for Kent which are not reproduced here.

The points which stand out in this table are : (i) the very great decrease in deaths after the 1843–1855 gap ; (ii) the higher incidence in females ; and (iii) the great increase in hydatid mortality during the years 1864–1880.

Summary

Mortality in England and Wales from helminth infections as recorded in the London Bills of Mortality from 1630 to 1755 and in the Registrar-General's Annual Reports from 1837 to 1880 is analysed.

References

Bills of Mortality, 1759.—"A collection of the yearly Bills of Mortality from 1657 to 1758." London.

Graunt, J., 1676.—"Natural and political observations mentioned in a following index and made upon the Bills of Mortality." 5th edition. London.

Registrar-General, 1837–1880.—"Annual reports." London.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 85–92.

A British Root-knot Nematode, *Meloidogyne artiellia* n.sp.

By Mary T. Franklin

Rothamsted Experimental Station, Harpenden, England

Roots of oats grown on sandy loam in Norfolk were observed by E. B. Brown of the National Agricultural Advisory Service, Cambridge, to have small galls associated with proliferation of the lateral roots. Close examination showed the presence of female *Meloidogyne* in the galls. The nematodes differed in some respects from the generally-known species and specimens were sent to the author for study.

Tests by Brown showed the following plants to be hosts : Kale (*Brassica oleracea* v. *acephala*), swede (*B. napus* v. *napobrassica*), pea (*Pisum sativum*), bean (*Vicia faba*), clover (*Trifolium pratense*), lucerne (*Medicago sativa*) : oats, wheat and barley were lightly infested. I have found cabbage (*Brassica oleracea* v. *capitata*), Brussels sprouts (*B. oleracea* v. *gemmifera*) and *Medicago lupulina* to be infested. In the last plant mature females were seen in the bacterial nodules. Brown found no infestation on grasses, carrots, sugar beet, tomato, onion, strawberry, lettuce, potato, sainfoin and rye. Dwarf bean (*Phaseolus vulgaris*) was immune when tested by me.

In January, 1958, kale growing in a different field on the same farm was found by Brown with the same *Meloidogyne* in the roots. The very small galls each had a single flask-shaped female partly embedded in the tissue and enveloped in a gelatinous egg sac (Fig. 1A).

From the roots of cabbages grown in soil from the infested area specimens of all stages of the nematode were obtained. The differences in the posterior cuticular pattern of the females, as well as in other characters, appear sufficient to separate this population from all the previously described species and it is here described as *Meloidogyne artiellia* n.sp.

The nematodes were fixed in the roots by immersion in hot cotton blue lactophenol and measurements were made on specimens mounted in the same medium and drawn under the camera lucida, unless otherwise stated.

MATURE FEMALE

Length :	0.7 mm.	(0·76—0·65 mm.)	(n=8)
Breadth :	0·4 mm.	(0·46—0·34 mm.)	(n=8)
Stylet :	14μ	(12—16μ)	(n=10)

Body swollen, pear- or flask-shaped, tapering gradually anteriorly to a small head ; smooth, rounded posteriorly, with terminal vulva. Annules visible in neck region and around tail.

As seen in Fig. 1A, the females that develop in brassica roots are usually only partly embedded in the small galls. The exposed part of the body is completely enveloped by the gelatinous material into which the eggs are laid ; this condition, which occurs in young females even before eggs are produced, is useful in distinguishing this *Meloidogyne* from young females of *Heterodera cruciferae*, which may be found side by side on the same roots. Young *Heterodera* females have a small gelatinous egg sack which enlarges as the eggs are laid and is therefore always well filled with eggs. *Heterodera* can also be distinguished by its small vulval cone, while in *Meloidogyne* the vulva is not prominent. Brown has found specimens of the two genera on the same oat plant, the cyst-forming species in this case being *H. major* (=*avenae*).

The broad "neck" narrows abruptly at the head which is 4–5μ across. In face view there appear to be six almost equal lips, and a small labial cap around the mouth aperture. The amphids open as short slits on the inner edge of the lateral lips. Each of the four sub-lateral lips has a small papilla, but none was visible on the lateral lips (Fig. 1C). Optical sections show a delicate, six-radiate skeletal structure around the anterior end of the stylet, but it disappears below the level of the lips (Fig. 1D). Dorsal views of the head show a constriction on the lateral lips about one-third behind the anterior edge. These lips could therefore be described as consisting of two unequal annules (Fig. 1E). The excretory pore lies ventrally one or two stylet lengths behind the head.

The stylet is very slender and may be slightly curved ; it has well developed rounded knobs, somewhat triangular in side view. The duct of the dorsal oesophageal gland opens into the lumen of the oesophagus 4–7μ behind the stylet base. The swollen pro-corpus is constricted at its junction with the large, muscular corpus (Fig.1F). The lobes of the oesophageal gland overlie the intestine latero-ventrally. The two ovaries are coiled around the swollen intestine, often extending into the neck, and the oocytes are in several rows. The six rectal glands described by Maggenti and Allen (1960) are visible in young females viewed from the caudal end.

The cuticular pattern round the vulva and anus is characteristic

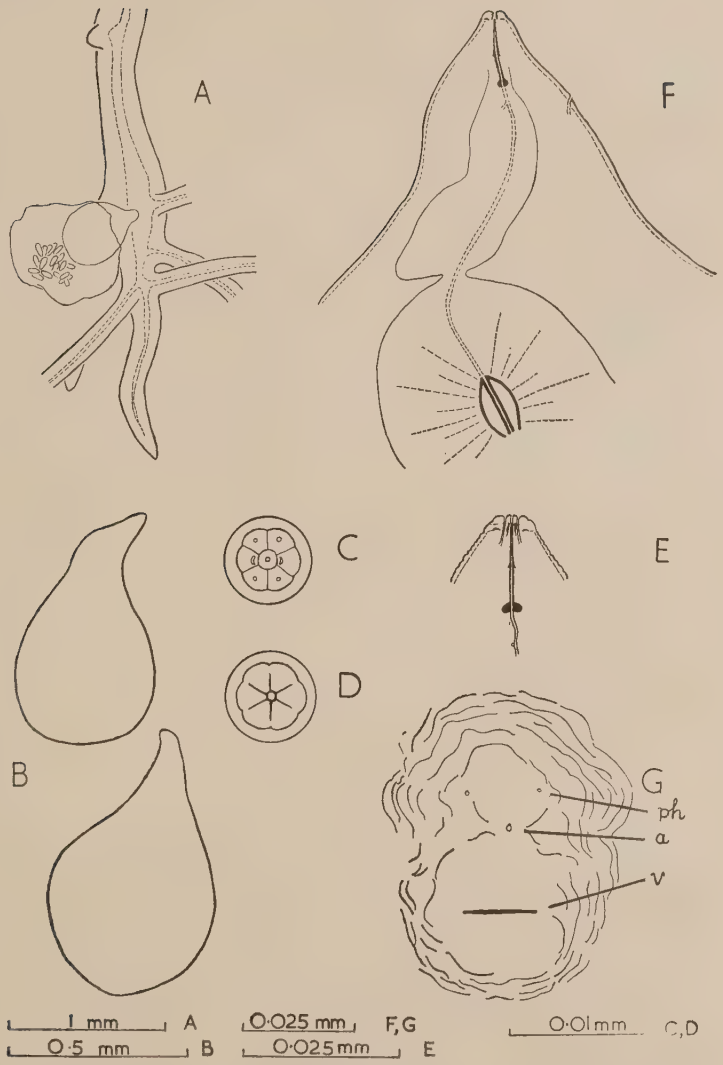


Fig. 1.—*Meloidogyne artiellia* n.sp. female

A.—Cabbage root with mature female. B.—Shape of females. C.—Head in face view. D.—Head skeleton. E.—Dorsoventral view of head. F.—Oesophageal region. G.—Posterior cuticular pattern.

(Fig. 1G and Plate I). It is formed of striae and ridges of the cuticle, the latter being more pronounced nearer the vulva and anus. In general outline the pattern is roughly that of a figure eight, the upper, smaller area enclosing the phasmids which are usually quite distinct, the anus situated at the centre and the vulva occupying the diameter of the lower, larger part of the pattern. At the top of the arch, which is morphologically the dorsal part of the tail, the pattern is usually angular. Cuticular folds curve towards the anus from each side, but leave a smooth unpatterned area around the vulva. The vulva is further from the anus in relation to the tail length than in most other species of the genus. The distance from anus to vulva is about three times that from the anus to a line joining the phasmids. The exact position of the tail tip is difficult to determine because the lateral lines are marked only by the position of the phasmids and by slight irregularities in the striae. The vulva is 15–22μ wide, with a mean for ten specimens of 17·5μ. In all but two of the specimens measured, the vulva is slightly wider than the distance apart of the phasmids.

Eggs

95μ × 37μ (75–111μ × 34–43μ) (n=20)

Male

Length 1·07 mm. (0·82–1·37) (n=15)
Breadth 29μ (23–36μ) (n=15)
a=35 (31–40)
*b 12·8 (10–15) (n=14)
c=83 (60–100) (n=10)
Testis 60% (45–76%) (n=15), occasionally reflexed
Spicules 27μ (25–30μ) (n=12)
Gubernaculum 8μ (6–9μ) (n=10)
Stylet 19μ (17–27μ) (n=7)

Body annulated, annules about 1·5μ wide. Lateral fields with four incisures at the tail, but along the greater part of their length a fifth incisure is present in the centre of each field (Fig. 2B). The lateral fields continue round the tail which is twisted through about 90° (Fig. 2C). Phasmids small, approximately adanal.

Head with labial cap and six nearly equal lips. Face view shows the slit-like amphid openings on the lateral lips; papillae not seen, nor was a stellate skeletal structure, such as that in the female. In dorso-ventral view a constriction is seen on the lateral lips about one-third from the front (Figs. 2D & E). A tubular guide surrounds

* Measurements for the ratio b (total length divided by length of the oesophagus) were made from the anterior end to the posterior edge of the oesophageal bulb, as the end of the glandular region overlaps the intestine and is difficult to define.

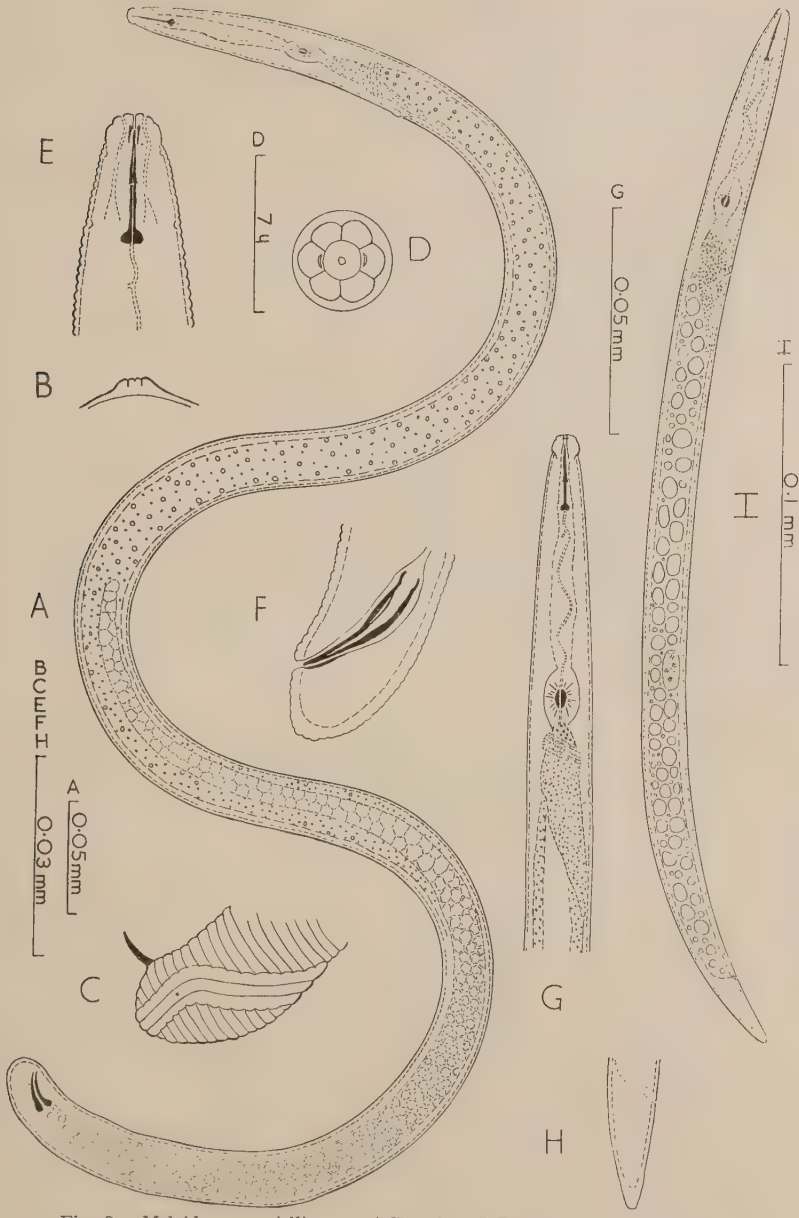


Fig. 2.—*Meloidogyne artiellia* n.sp. A-F male: G–I.—Second stage larva.
A.—Whole male. B.—Transverse section of lateral field. C.—Tail surface.
D.—Face view of head. E.—Dorsoventral view of head. F.—Spicules.
G.—Oesophageal region. H.—Tail. I.—Whole larva.

the anterior end of the stylet which has well-developed, rounded, basal knobs. The duct of the dorsal oesophageal gland opens into the lumen of the oesophagus 5–7μ behind the stylet. Posterior cephalids (Hirschmann, 1959) are situated at about the level of the anterior edge of the stylet knobs ; at this point the body narrows perceptibly forming a neck-like region. Anterior cephalids are just discernible immediately behind the head constriction (Fig. 2E).

Pro-corpus narrow, two to three body-widths long, followed by a spindle-shaped muscular corpus about twice as long as wide. The oesophageal glands stretch for about three body-widths ventro-laterally along the intestine. Nerve ring one bulb-length behind muscular bulb. Two body-widths behind the oesophageal bulb is a conspicuous hemizonid and immediately behind it is the excretory pore with its duct running back for a short distance (Fig. 2A).

Spicules typical for the genus, curved with anterior thicker part and tapering posteriorly to a point. A small gubernaculum, about one-third the length of the spicules, lies dorsally in the cloaca wall. Tail very slightly longer than the anal body diameter (Fig. 2F).

Testis about two thirds of the body length and sometimes reflexed anteriorly. Of about twenty specimens only one had two testes. There was nothing to indicate whether any correlation existed between number of testes and nutrition of the nematode during development, as shown by Triantaphyllou (1959) to be the case in *M. incognita.*

LARVA

Length 354μ (334–370μ) (n=20)
Breadth 15·4μ (10–16μ) (n=20)
a 23·5 (22–25·5) (n=20)
**b* 5·9 (5·4–7) (n=20)
c 14·3 (13–16) (n=11)
Stylet 14·7μ (14–16μ) (n=10)

The above measurements were made on larvae (presumed to be second-stage) hatched from egg masses in water, fixed in T.A.F. and transferred to warm cotton blue lactophenol, in which they were mounted. The most striking feature of the larvae is the short tail with rounded tip. It is about 24·5μ long, and two and one half times as long as the body diameter at the anus (Fig. 2H). Body finely annulated with lateral fields about one third of the body width wide and marked by four longitudinal incisures for most of their length. At the level of the oesophageal bulb the two inner incisures merge and

* Measurements for the ratio *b* (total length divided by length of the oesophagus) were made from the anterior end to the posterior edge of the oesophageal bulb, as the end of the glandular region overlaps the intestine and is difficult to define.

disappear leaving the outer two which coincide and disappear close behind the stylet. On the tail the lateral fields narrow gradually behind the anus and disappear close to the tip. Phasmids small, situated at about one third of the distance between anus and tail tip. No deirids observed.

The head has a shallow cap and in lateral view no annules are visible on the lips.

Stylet unusually long and very slender. Duct of dorsal oesophageal gland opening 3·7μ (2·5–4·5μ, $n=10$) behind stylet knobs, which are rounded, triangular as in the female.

Procorpus narrow, leading to well-developed muscular bulb with the usual three valve plates in the centre. Bulb followed by a narrow isthmus about one body-width long which broadens into the intestine. Nerve ring encircles isthmus about one bulb-length behind muscular bulb and just in front of the hemizonid. The glandular lobes lie along the intestine latero-ventrally for about two body-widths. The hemizonid, with the excretory pore and duct very close behind it, is nearly two body-widths behind the muscular bulb (Fig. 2G).

Head offset by a slight constriction; labial annule appears smooth and labial cap very shallow. Immediately behind the head constriction an anterior cephalid is just visible and a posterior one is present close in front of the level of the stylet knobs. There is no marked head skeleton.

DIAGNOSIS. *Meloidogyne artiellia* has most of the characters of the genus but differs from all other described species in the posterior cuticular pattern of the female and in the tail and stylet of the newly hatched (i.e. second stage) larva. Cuticular pattern marked by coarse, angular ridges round anus and vulva, squarish dorsally and bending towards anus from either side. Lateral fields not readily traceable; phasmids close together (about vulval-width apart); anus nearer to tail tip than to vulva. Outer part of pattern formed from fine, fairly smooth striae.

Larval stylet longer (14·7μ) than that of any species except *M. africana* (12–18μ) and *M. brevicauda* (14·3–14·5μ). Larval tail short and blunt: the only other blunt-tailed species, *M. brevicauda*, has a very different posterior cuticular pattern, and in the male the two subventral and two subdorsal lips are fused. *M. brevicauda* also has a short-tailed larva, as does *M. africana*; all except one other species, apart from the new one, have longer tails ($c=10$). The exception, *M. inornata*, has $c=11·6–12·7$, but the tail is sharply pointed.

In view of the characters shown by this species and by *M. africana* (Whitehead, 1959) and *M. brevicauda* (Loos, 1953), three slight amendments should be made to the generic diagnosis given by Allen (1952):

(a) Six lips, but sub-dorsals and sub-ventrals may be fused (*M. brevicauda*).
(b) Lateral lips not always markedly larger than sub-medians (*M. artiellia*).
(c) Anus and vulva sometimes on an eminence (*M. africana*).

Holotype Female, slide number 77/8/1
Length 0·7 mm.
Breadth 0·4 mm.
Stylet 14μ

Allotype Male, slide number 77/8/2
Length 0·952 mm.
Breadth 41μ
Stylet 17μ
Spicules 28μ
$a=32$; $b=13$; $c=92$; T=65%

Type Host—Roots of Cabbage, *Brassica oleracea capitata*
Type Locality—Wells, Norfolk, England.
Allotypes on slides nos. 77/8/3–7 Nematology Collection, Rothamsted Experimental Station.

This is the first species of *Meloidogyne* to be described initially from Britain. *M. hapla* has been found on field crops on several occasions (Brown, 1955) and I have seen it on *Succisa pratensis* Moench and *Ajuga reptans* L. from Northumberland. It seems highly probable that it also is an indigenous species. The widely-held view that root-knot eelworms are found in the field exclusively in tropical and sub-tropical regions, and occur in northern Europe only in glasshouses, must therefore be modified. It may well be that root-knot disease occurs more often in the field in temperate climates than has hitherto been realised.

References

Allen, M. W., 1952.—"Observations on the genus *Meloidogyne* Goeldi, 1887." *Proc. helminth. Soc. Wash.*, **19** (1), 44–51. (W.L. 16747a).

Brown, E. B., 1955.—"Occurrence of the root-knot eelworm, *Meloidogyne hapla*, out of doors in Great Britain. *Nature, Lond.*, **175** (4453), 430–431. [Correspondence] (W.L. 14900).

Hirschmann, H., 1959.—"Histological studies on the anterior region of *Heterodera glycines* and *Hoplolaimus tylenchiformis* (Nematoda, Tylenchida)." *Proc. helminth. Soc. Wash.*, **26** (2), 73–90. (W.L. 16747a.)

Loos, C. A., 1953.—"*Meloidogyne brevicauda*, n.sp. a cause of root-knot of mature tea in Ceylon." *Ibidem*, **20** (2), 83–91.

Maggenti, A. R. and Allen, M. W., 1960.—"The origin of the gelatinous matrix in *Meloidogyne*." *Ibidem*, **27** (1), 4–10.

Triantaphyllou, T., 1959.—"Variation, post-infection development and sex determination in *Meloidogyne incognita* and oogenesis in some *Meloidogyne* species." *Thesis, North Carolina State College.*

Whitehead, A. G., 1959.—"The root-knot nematodes of East Africa. I. *Meloidogyne africana* n.sp., a parasite of arabica coffee (*Coffea arabica* L.)." *Nematologica*, **4** (4), 272–279. (Published January, 1960).

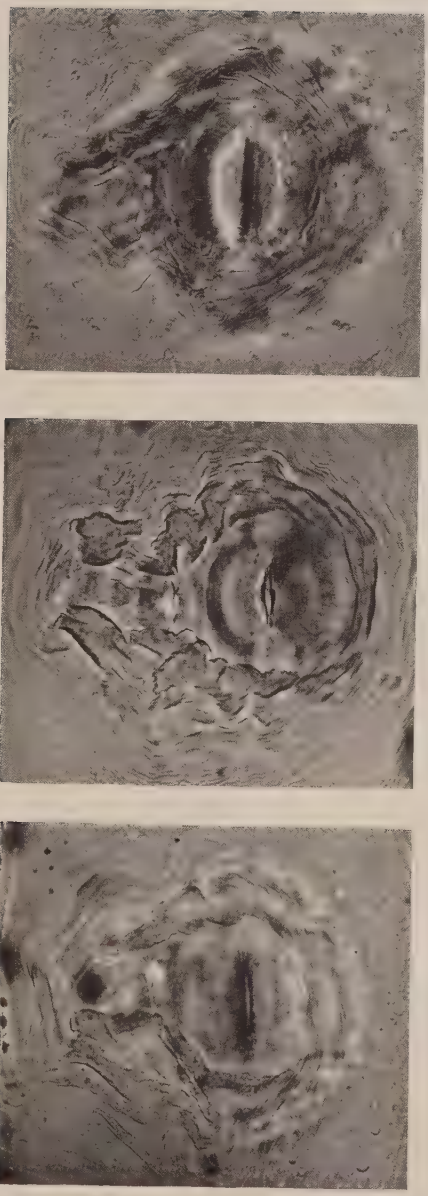


Plate.—Posterior cuticular patterns of three female *Meloidogyne artiellia* n.sp.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 93-100.

Hormones Corticotropes et Cortico-surrénales dans les Infections à Helminthes

Par Henri Galliard
Faculté de Médecine de Paris, Institut de Parasitologie

Les conceptions concernant les propriétées de la cortisone et l'ACTH ont beaucoup évolué. On considérait ces hormones comme nuisibles dans les infections bactériennes et parasitaires puisque leur principale propriété est de neutraliser la réaction inflammatoire, histiocytaire et macrophagique. Or dans beaucoup de cas on a observé des résultats favorables dans différentes infections, et on a fait remarquer que plus le processus septicémique est sévère, meilleur est l'effet obtenu. Dans les infections à protozoaires sanguicoles les résultats sont extrèmement divergents même chez l'homme.

Quant aux maladies dues aux helminthes peu d'entre elles présentent un caractère de gravité. Les épisodes inflammatoires aigus sont exceptionnels sauf dans les distomatoses hépatiques dans les bilharzioses et surtout la trichinose.

Il est bien évident que l'ACTH et la cortisone n'ont aucun effet parasiticide, mais c'est par leur propriétés anti-inflammatoires, anti-allergiques, qu'elles peuvent agir au cours d'un épisode aigu et sauver un malade.

A.C.T.H. et cortisone dans les infections expérimentales.—La trichine se prête particulièrement bien à l'étude experimentale des effets de l'ACTH et de la cortisone sur l'immunité dans les helminthiases. C'est un parasite qui s'adapte à tous les mammifères sans distinction.

Pour Stoner et Goodwin (1954), C. M. Coker (1955), Markell et Lewis (1957), R. A. Lord (1958) l'ACTH accroît légèrement la réceptivité de la souris pour la trichine. La cortisone au contraire provoque une réceptivité beaucoup plus grande et une mortalité beaucoup plus élevée que chez les témoins. L'éosinophilie n'apparaît pas chez les souris traitées par la cortisone. De plus la cortisone inhibe la résistance acquise par une primo-infestation. Par contre l'ACTH chez les souris immunisées et réinfestées ne provoque pas

une augmentation significative de la mortalité. Quand les souris sont déjà immunisées par une primo-infestation la cortisone supprime cette immunité et empêche, d'autre part, les souris neuves, d'acquérir la résistance à la surinfection comme les témoins non traités.

Quoiqu'il en soit ces experiences montrent que l'ACTH doit être utilisé à l'exclusion de la cortisone dans la trichinose humaine.

Dans le cas de *Schistosoma mansoni*, Coker (1957 et 1958) a montré que la cortisone chez la souris n'a pas semblé avoir augmenté la réceptivité de l'animal. Pour Weissman et Hunter (1959) on peut estimer que les souris traitées sont moins parasitées que les témoins. La cortisone a souvent un effect toxique dans la bilharziose expérimentale (Nagaty *et al.* 1956).

La trichine est un parasite qui s'adapte à tous les mammifères mais d'autres présentent une spécificité très stricte pour un hôte donné. Dans ce cas la cortisone et l'ACTH peuvent supprimer cette spécificité et rendre sensible des animaux normalement réfractaires. De même ces hormones peuvent annuler l'immunité conférée par l'âge et créer chez les animaux âgés le terrain réceptif des jeunes.

H. Galliard et Berdonneau (1953) ont montré l'effet de la cortisone sur la réceptivité du chien qui est parfois réfractaire à la strongyloïdose humaine. Il y a eu dans deux cas un effet certain de la cortisone.

E. Roman (1956) avec la cortisone a réussi à infecter des souris blanches, habituellement réfractaires, avec *Strongyloïdes ratti*.

En ce qui concerne les filaridés, on sait que le rat blanc présente un état réfractaire absolu à l'infection par *Litomosoïdes carinii* parasite du "cotton-rat". N. T. Byggs (1959), Olson (1959) ont montré que la cortisone supprime cette immunité naturelle.

E. Roman (1958) en traitant des rats et des souris âgées avec l'acétate de cortisone préalablement pendant une semaine et pendant toute la durée de l'observation, a pu les infester avec le cestode *Hymenolepis fraterna*, comme si l'hormone avait créé chez eux un terrain de jeunes.

Résultats cliniques.—Il est une maladie parasitaire qui a bénéficié au premier chef de la thérapeutique hormonale-c'est la trichinose. Après bien des essais et des déboires on a renoncé à la thérapeutique spécifique. Certes avec l'Hetrazan-Notézine on avait fait quelques progrès.

Davis et Most, les premiers, ont utilisé l'ACTH dans le traitement de la trichinose aigüe dans un cas très grave. Il y eut une amélioration remarquable de l'état général et une sédation presque complète des

symptômes : fièvre, vomissements, inappétence absolue, oedèmes de la face et des extrémités, état confusionnel ; délire, excitation. Seul persista un état de fatigue intense, d'épuisement. Le taux d'éosinophilie qui était de 45% au début du traitement, tomba à 5% vers le 17e jour. Malgré la persistance des larves vivantes, la myosite et l'infiltration cellulaire, l'amelioration clinique observée au cours du traitment persista. Le bénéfice du traitment est donc à attribuer, non à une action parasiticide mais à des effets non spécifiques, anti-allergiques.

Les autres auteurs qui ont utilisé les hormones, Luongo avec l'ACTH, Khalily, Rosen avec la cortisone, ont fait des observations semblables. Les symptômes les plus graves, comme la myocardite, cèdent en quelques heures. La température tombe, les douleurs cessent, l'état général s'améliore. L'éosinophilie remonte après arrêt du traitment, mais sans qu'il y ait modifications des symptômes ni hausse de température.

On peut constater aussi que les hormones n'interviennent pas sur le développement de l'immunité. Il y a en effet chez les malades traités, une élévation progressive du taux des anticorps. Par contre, comme on peut s'y attendre, il y a suppression de l'allergie cutanée et les tests donnant des résultats négatifs.

Il est intéressant de constater à propos de la trichine et des hormones hypophysaires et cortico-surrénale, le divorce entre la clinique humaine et l'expérimentation. Chez la souris elles rendent le sujet plus réceptif à l'infestation et le parasitisme plus intense ; elle suprime l'immunité acquise par une primo-infestation et elles empêchent les souris neuves d'acquérir l'immunité. Mais la trichinose est une maladie où l'on peut négliger le parasite, mais on sauve le malade en traitant les symptômes.

Il est évident que toutes les affections dues aux helminthes, surtout les trématodes et les nématodes qui sont les plus allergisants, peuvent bénéficier du traitement par l'ACTH et la cortisone. C'est le cas des schistosomoses ou bilharzioses. On observe en effet assez fréquemment des crises aigües avec fièvre élevée, atteinte grave de l'état général qui ne répondent absolument pas au traitement antimonié. Koang, Tung et Tchén (1956) ont rapporté 13 cas d'individus ayant une infection probablement récente, présentant tous des oeufs dans les selles et qui souffraient de fièvre élevée à 40°, cyanose, hépatomégalie et splénomégalie, douleurs abdominales, toux, ombres labiles visible à la radiographie. Avec l'ACTH, même dans un cas particuliérement grave, la fiévre tomba en 3 jours. Enfin l'ACTH a permis de commencer le traitement antimonié beaucoup plus précocement à la période fébrile, sans danger.

Effets de l'ACTH et la cortisone sur l'éosinophilie d'origine parasitaire.—On sait que l'hypéréosinophilie est fréquente en dehors des helminthiases dans les leucémies à éosinophiles dans les processus toxiques et irritatifs tels que certaines intoxications, l'asthme, les dermatoses, la résorption d'épanchements, l'injection d'antibiotiques, la radiothérapie ou au cours des maladies infectieuses.

On dit qu'il y a éosinophilie quand le nombre absolu des cellules éosinophiles du sang circulant dépassant environ 200 éosinophiles par mm 3 de sang circulant, 0·5 à 2% environ.

On sait que la stimulation de la cortico-surrénale provoque de nombreuses modifications métaboliques ou autres, qui se font par l'intermédiaire de la sécrétion des corticoïdes du type de l'hydrocortisone et dont la plus apparente est l'éosinopénie. L'ACTH, la corticostimuline excitant physiologique de la surrénale, est le type des substances éosinopéniantes. L'éosinopénie est donc un signe d'hypercorticisme.

Ainsi l'irréductibilite de l'éosinophilie observée souvent aprés injection d'ACTH s'explique dans une certaine mesure puisque cette hormone doit agir sur un cortex surrénal normal pour provoquer l'éosinopénie par sécrétion de cortisone. Par contre l'injection de cortisone devrait toujours provoquer l'éosinopénie tout au moins temporairement.

On a cherché à savoir d'une part s'il était possible de distinguer les éosinophilies d'origine parasitaire d'après les effets de l'ACTH et de la cortisone.

Mais il faut distinguer évidemment un test comme celui de Thorn classique et les doses thérapeutiques dont les effects sont analogues à ceux du test de Thorn prolongé à l'ACTH retard (ACTH zinc, ou gélatine, ou polyphloretol) et surtout les tests intraveineux qui réalisent une stimulation progressive et prolongée.

Nous avons dit que dans la trichinose grave ou la bilharziose aigüe traitée par l'ACTH il y a toujours réduction de l'éosinophilie pendant toute la durée du traitement et remontée de la courbe aux taux supérieurs ensuite, malgré la cessation de la fièvre et des autres symptômes de la phase aigüe, sans que l'état clinique s'en ressente. (Luongo, Reid et Weiss, 1951, Davis et Most 1951). Warter et Moïse (1953) dans un cas de *Loa loa* ont vu l'éosinpohilie passer de 5,140 à 820 au bout de huit heures.

En ce qui concerne la cortisone, Deschiens et Mauzé (1952) à la Guadeloupe, Deschiens, Lamy et Estival (1953), par un traitement continue (700 mgr. en 20 jours) ont constaté l'irréductibilité du

nombre des éosinophiles dans un cas d'anguillulose et un d'ascaridiose, et l'éosinopénie dans les cas d'éosinophilie dite tropicale.

Chez des Africains nous avons observé que l'effet de la cortisone 200 mgr. par jour, per os, était extrèmement variable. Par contre dans six cas d'eosinophilie chez des européens la cortisone a provoqué toujours une éosinopénie considérable tant que le traitement persiste avec remontée de la courbe aussitôt après.

Dans le cas de *larva migrans viscérale* de Dent, Nichols, Beaver, Carrera et Staggers (1956) l'enfant fut maintenu pendant 40 jours sous effet de la cortisone, 150 mgr. par jour per os. Le deuxième pour l'éosinophilie tombe de 54% à 4% et ce taux persiste jusqu'à l'arrêt du traitement. La courbe remonte alors neuf jours après à 32%.

En ci qui concerne l'hypéréosinophilie dûe aux extraits d'helminthes, Studer et Fust (1951) ont montré que la cortisone pouvait réduire l'hyper-éosinophilie et neutraliser la sensibilisation obtenue par des extraits d'ascaris chez le cobaye. Par contre Deschiens, Poirier et Lamy (1953) ont constatés que chez cinq cobayes sensibilisés par l'extrait de *Fasciola hépatica*, l'éosinophilie n'éntait pas réductible par les injections de cortisone, ni par l'ACTH.

Test de Thorn.—Le test de Thorn se fait avec 25 mgr. d'ACTH par voie intramusculaire et numération de l'éosinophilie après 4 heures. Le test est normal, positif s'il y a labilité de l'éosinophilie ; négatif si elle est irréductible. F. Delbarre semble avoir été le premier à signaler en 1950 un cas de "filariose" décelée par intradermo-réaction où l'éosinophilie fut irréductible. Telkka, Wahlstrom, Koulumies en 1951, en Finlande, chez 33 individus, porteurs de bothriocéphales, ont vu dans la majorité des cas, une chute de 50% (test positif).

Nous avons signalé que Luongo, Reid et Weiss avaient obtenu en 4 heures avec 10 mgr. d'ACTH une éosinopénie considérable. Mollaret dans un cas de trichinose, a trouvé un résultat négatif avec chute des éosinophilies de 29% seulement. Warter et Moïse (1953) ont obtenu, dans deux cas de *Loa loa*, deux tests négatifs. Signalons enfin que Morales, Casas et Sanz (1950), à Porto-Rico, ont utilisé l'adrénaline (0·3 mgr.) dans des cas de filariose (*W. bancrofti*), et de bilharziose (*S. mansoni*). Le test donna dans tous les cas un résultat nettement positif, ce qui est normal, puisque l'adrénaline, d'après les conceptions actuelles, agit directement sur les éosinophiles.

En 1953 H. Galliard, J. Lapierre, R. Berdonneau et M. Larivière ont pratiqué systématiquement le test de Thorn et ont trouvé dans 24 cas de parasitose une éosinophilie irréductible et dans 7 cas une éosinophilie labile.

Dans le cas de la strongyloïdose, les résultats ont été, sur six cas : trois négatifs, un douteux (chute de 45%) et un positif (chute de 56%). D'autre part, dans ces cas, il ne semble pas y avoir de rapport entre l'irréductibilité du nombre des éosinophiles et l'intensité ou l'ancienneté du parasitisme. Le degré d'éosinophilie n'intervient pas non plus. En ce qui concerne les autres parasitoses à Nématodes, Trichocéphale et *Ascaris*, les résultats étaient difficiles à interpréter.

C'est dans nos premiers cas de filariose à *Loa loa* que les résultats semblaient les plus probants, avec irréductibilité des plus nettes. Cependant chez deux sur six sujets africains le résultat du test fut nettement positif, (chute de 60%).

Il ne semble pas en outre qu'il y ait un rapport entre l'importance de l'éosinophilie et le sens du test de Thorn. Il n'y a pas non plus de rapport apparent avec l'ancienneté et l'intensité de l'infestation.

Nous nous sommes demandé, bien entendu, quelles pouvaient être les raisons de cette absence de réponse à l'ACTH dans certains cas de parasitose par helminthes. Il ne peut s'agir d'une insuffisance surrénale même modérée. Aussi chez une malade (*Loa loa*) qui avait présenté quatre fois une éosinophilie irréductible, une perfusion de 4 heures avec 10 mgr. d'ACTH a provoqué une éosinopénie considérable, puis retour au bout de 24 heures au chiffre antérieur. On peut rapprocher ceci du fait que Coste, Delbarre, Basset et Chabaud (1953) ont montré que chez un sujet considéré depuis dix ans comme addisonien, l'ACTH par perfusion a provoqué une éosinopénie.

On peut donc dire que, chez tous ces patients à éosinophilie irréductible, il y a non une insuffisance mais une paresse dans la réponse du cortex surrénal mais qui est vaincue par la perfusion.

Nos résultats ont montré que, dans l'ensemble de nos cas, ce test ne présentait pas une valeur certaine. Il semble que c'est nettement dans la filariose à *Loa loa* qu'il donne les résultats les plus constants.

Ultérieurement R. Deschiens et H. Floch (1954) ont obtenu chez 25 enfants antillais parasités, 92% de cas négatifs. De même Koerber (1954) au Sénégal, E. Montestruc et R. Berdonneau (1954) à la Martinique ont observé la fréquence de l'irréductibilité de l'éosinophilie, c'est à dire des tests à résultat négatif.

Résumé et Conclusions

L'étude expérimentale de la cortisone et de l'ACTH dans les infections à helminthes montre que leurs effets sont différents. Dans la trichinose les animaux traités par la cortisone, présentent une réceptivité accrue au parasitisme qui peut devenir considérable,

et ne contractent pas l'immunité solide acquise en quelques jours par les témoins. Les animaux solidement immunisés par une primo-infestation perdent complétement leur résistance à la surinfection.

L'ACTH au contraire a chez l'animal des effets analogues à ceux que l'on observe chez l'homme. Elle n'a pas l'effet toxique de la cortisone, elle ne rend pas les animaux plus sensibles, elle réduit efficacement la mortalité expérimentale, et malgré son effet anti-inflammatoire l'infiltration cellulaire restes suffisante pour obliger les larves à s'encapsuler alors qu'elles restent libres avec la cortisone.

Ces expériences ont donc l'intérêt de montrer qu'en thérapeutique humaine l'ACTH doit être utilisée à l'exclusion, pensent certains, de la cortisone, tout au moins dans la trichinose.

Expérimentalement la cortisone et l'ACTH peuvent dans une certaine mesure supprimer la spécificité d'un parasite pour un hôte donné et rendre sensible un hôte normalement réfractaire. Tel est le cas des Strongyloïdes humains qu'on peut adapter au chien, et celui du rat qu'on peut adapter à la souris ; il en est de même pour certaines filaires. On peut aussi, dans certains cas, neutraliser l'immunité d'âge en créant chez les animaux âgés le terrain réceptif des jeunes.

En clinique humaine l'ACTH et la cortisone n'ont aucune action parasiticide sur les helminthes, mais leur action anti-inflammatoire, anti-allergique rend les plus grands services. Dans la trichinose ces hormones permettent de sauver le malade dans les cas d'infestation grave qui étaient autrefois mortels. ACTH, surtout, supprime l'inflammation intestinale et musculaire de la phase aigüe souvent mortelle. Elle fait céder en quelques heures les symptômes les plus graves. Dans le cas des bilharzioses et des autres parasitoses les effets de la thérapeutique hormonale sont efficaces dans les épisodes aigüs.

L'éosinophilie d'origine parasitaire présente le caractère particulier d'être dans une certaine mesure rebelle à l'ACTH qui provoque normalement l'éosinopénie. Quant à la cortisone elle réduit toujours le taux de l'éosinophilie, mais il remonte toujours au chiffre antérieur dès cessation du traitement, ce qui est natural puisque la cause de l'éosinophilie persiste.

Enfin les manifestations cliniques et l'état de shock, ainsi que l'hyperéosinophilie, dus à l'injection et à la réinjection d'extraits parasitaires chez l'animal, sont réduits ou même supprimés par la cortisone.

Le test de Thorn classique (une seule injection intramusculaire de 25 mgr. d'ACTH et numération des éosinophiles au bout de 4 heures)

montre ce caractère rebelle aux faibles doses d'ACTH de l'éosinophilie parasitaire surtout dans les filarioses. Quoiqu'il en soit cette éosinophilie résistante à l'ACTH par voie intramusculaire peut toujours être réduite par l'injection intraveineuse, surtout par perfusion. Il n'y a donc, dans le cas des parasitoses, aucune lésion anatomique irréversible permettant d'expliquer cet état réfractaire aux éosinopéniants.

Bibliographie

Coker, C. M., 1956.—"Some effects of cortisone in mice with acquired immunity to *Trichinella spiralis*." *J. infect. Dis.*, **98**, 39–44. (W.L. 11250).

Davis, W. M. et Most, H., 1951.—"Trichinosis. Case report with observations of the effect of adrenocorticotropic hormone." *Amer. J. Med.*, **11**, 639–644. (W.L. 603b).

Delbarre, F., 1950.—"Les tests de Thorn." *Conf. Ecole nat. Santé.* (cité par H. Galliard, 1953.)

——— et Loyau, G., 1957.—"Valeur du test hématologique de Thorn." *Sang*, **38**, 528. (W.L. 19704).

Deschiens, R., Lamy, L. et Estival, J., 1953.—"Action de la cortisone dans l'éosinophilie parasitaire." *Bull. Acad. nat. Méd.*, **137**, 308. (W.L. 3685).

——— et Mauzé, J., 1953.—"Action de la cortisone dans l'éosinophilie tropicale." *Bull. soc. Pat.exot.*, **46**, 214. (W.L. 5310).

——— et Poirier, M., 1953.—"Etude comparée des propriétés toxiques et éosinophilogènes de différents extraits de douves chez le cobaye." *C. R. Soc. Biol. Paris*, **147**, 1059–1061. (W.L. 6630).

Galliard, H., 1954.—"L'éosinophilie dans les filarioses." *Riv. Parassit.*, **15**, 403–423. (W.L. 19442a).

———, 1959.—"Trichinose." *Encycl. Médic. Chir.*, (Maladies infectieuses 8115/A^{10}).

——— et Berdonneau, R., 1953.—"Strongyloidose expérimentale chez le chien. Effets de la cortisone. Résultats du test de Thorn à l'hormone corticotrope ACTH." *Ann. Parasit.*, **28**, 163–171. (W.L. 899a).

———, Lapierre, J., Berdonneau, R. et Larivière, M., 1953.—"Effets de l'ACTH et la cortisone sur l'évolution et l'éosinophilie dans certaines infestations à helminthes, humaines et expérimentales." *C. R. Acad. Sci.*, **236**, 639–640.

———, Lapierre, J., Larivière, M., et Berdonneau, R., 1953.—"Test de Thorn à l'ACTH et autres épreuves de fonctionnement cortico-surrénal dans des cas d'infestations par les helminthes." *Ann. Parasit.*, **28**, 372–386.

Lord, R. A., 1958.—"Studies on the use of cortisone and ACTH in Trichinosis." *Amer. J. trop. Med. Hyg.*, **7**, 611–617.

Luongo, M. A., Reid, D. H. et Weiss, W. S., 1951.—"The effect of ACTH in trichinosis." *New Eng. J. Med.*, **245**, 757. (W.L. 15073a).

Markell, E. K. et Lewis, W. P., 1957.—"Effect of cortisone treatment on immunity to subsequent reinfection with *Trichinella* in the rat." *Amer. J. trop. Med. Hyg.*, **6**, 553.

Rosen, E., 1952.—"Cortisone treatment of trichinosis." *Amer. J. med. Sci.*, **223**, 16–19. (W.L. 603).

Stoner, R. D. et Godwin, J. T., 1953.—"The effects of ACTH and cortisone upon susceptibility to trichinosis in mice." *Amer. J. Path.*, **29**, 943–950. (W.L. 608b).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 101–106.

The Nature of the Spear Guiding Apparatus in Dorylaimidae

By J. Basil Goodey
Rothamsted Experimental Station, Harpenden

Confusion exists about the true nature of the guiding apparatus surrounding the spear in Dorylaims. Thorne and Swanger (1936), commenting on the guiding ring, say : "There are at least two types ; the collapsible cylindrical form with cutinized borders that give the appearance of a "double" ring such as that of *Dorylaimus stagnalis* (Fig. 2A), and the single muscular ring as found in *D. obscurus* (Fig. 2E). From the guiding ring a thin sheath envelops the spear to its base."

Three years later Thorne (1939) elaborating and modifying this statement said : "Two types of guiding ring are present in the Dorylaimoidea. The 'double' type is that observed in *Labronema* (essentially similar to Fig. 2A, present paper) and many other forms (Pl. 1, Fig. 7). This consists of a strongly cuticularized basal ring placed well back in the pharynx with a smaller ring in front of it, the two joined by a collapsible sleeve-like membrane which allows the front ring to extend far forwards as the spear is extended or, as the spear is retracted completely, draws the front ring entirely within the basal ring in which condition the ring may be said to be 'collapsed'. The 'single' type consists of a muscular collar which is somewhat extensible but which does not give the 'double' optical appearance (Pl. III, Fig. 19) (essentially similar to Fig. 1A, present paper)."

Recent examination of live specimens of various Dorylaim genera indicates that the guiding apparatus in all has the same basic structure. The guiding apparatus is essentially a sheath fixed in three places ; anteriorly it is fused with the walls of the stoma, forming the anterior fixed ring ; medianly there is another fusion with the stoma wall and posteriorly the sheath is fused round the base of the spear at about its junction with the spear extension. The first and median junctions are outward attachments, the basal junction inward (Fig. 1A). In most specimens the clearance between stoma wall and spear is so narrow that the median and basal attachments are very difficult to see and their nature can usually only be inferred from slight indications.

In living specimens, when the spear is being moved back and forth, the depth of that part of the sheath projecting beyond the anterior fixed ring varies ; when the spear is retracted, only the fixed anterior ring shows—the "single" state—, but as the spear is protruded the guiding sheath evaginates till, when the spear is fully protruded, a broad band or two rings appear—the "double" state. Figs. 1A, B & C show this progressive variation in a Dorylaim. In what may be called "the position of rest", that is the position in which the spear is held in the body when not being used, some species show a "single" ring others a "double" one. The appearance of this region, however, varies from specimen to specimen in the same species.

Thorne's application of the terms "cutinized", "muscular" and "cuticularized" to the guiding ring is misleading. All three terms imply a solidity and fixity that the anterior end of the guiding sheath of living specimens lacks.

The literature contains little on the variation shown by the anterior part of the guiding sheath, but the state of a "single" or "double" guiding ring is commonly used as a diagnostic criterion. However, many specimens from many species will need to be studied to show whether it can serve for diagnosis, for it seems to be a variable character.

In the figures published by Thorne and Swanger (1936) and Thorne (1939) of the Dorylaims with axial spears, the depth of the guiding ring, that is of the forwardly evaginated part of the guiding sheath beyond the anterior fixed ring, seems correlated with spear position and the deepest rings are seen when the spear projects from the stoma. Also, none of the figures shows the thin sheath mentioned by Thorne and Swanger as surrounding the spear.

Goodey, Peacock and Pitcher (1960) showed a positive correlation between length of guiding sheath and position of spear in *Xiphinema diversicaudatum*, but the current interpretation of the nature of the guiding sheath was not then clear. At moulting, the lining of the stoma, the guiding sheath and the lining of the spear extension, are shed together with the spear. Further examination of moulting specimens now shows the double-walled nature of the forwardly evaginated part of the guiding sheath projecting beyond the anterior fixed ring, and the continuation backwards, behind it, of a single fine sheath surrounding the spear.

Thorne and Swanger (1936) discussed the characterization of *Aporcelaimus* and said : *"guiding ring for spear absent* but plications of pharynx sometimes give appearance of guiding ring." Altherr (1952) pointed out that this determination depended on the observer and on the state of preservation of the nematode. Williams (1959)

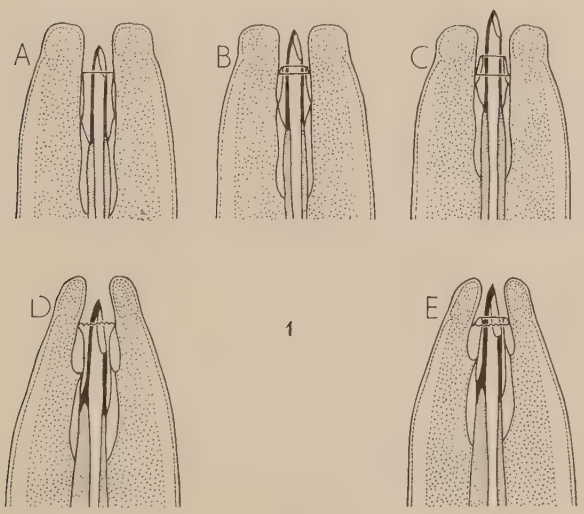


Fig. 1. A, B & C.—*Dorylaimus* sp.—stages in the evagination of the guiding sheath ; A. the "single" state, anterior fixed ring the most forward part ; B & C. different types of the "double" state of the guiding ring, sheath evaginated beyond the anterior fixed ring. D & E. *Aporcelaimus* sp.—D. plicated, anterior fixed ring ; E. evagination of sheath beyond the fixed ring seen in a living specimen. N.B.—All figures are diagrams.

says that the mouth of the guiding sheath is wide and irregular and not clearly ring-like, but his descriptions and figures show the sheath to be usually free and projecting forwards into the stoma. This is a common interpretation of the anterior end of the guiding sheath because the wall of the anterior part of the stoma is very faint and difficult to see in fixed and mounted specimens. In live specimens

the expanded forward edge of the sheath is clearly fused with the stoma walls and is, in fact, the anterior fixed ring described above (Fig. 1D). It is not very outstanding optically. I have observed in live specimens of *Aporcelaimus*, as the spear moved forwards, that an evagination of the guiding sheath also came forward and projected beyond the plicated anterior fixed ring (Fig. 1D and E). The relationship of guiding ring to stoma and spear showed well in a specimen of *A. minor* Loos, 1945 (Fig. 2B).

The definition of *Aporcelaimus* needs emendation to allow for this new interpretation of the nature of the guiding sheath. The anterior end of the guiding sheath is somewhat funnel-shaped, its margin often somewhat irregular or plicated, but fused to the stoma wall as a fixed anterior ring in the "single" state. About halfway down its length the sheath is fused again to the stoma wall and at its base is fused "inwardly" around the spear base.

The Nygolaiminae are characterised by the spear being a mural tooth on the left subventral wall of the stoma (in *Oionchus* it is said to be dorsal). There are plications of the stoma wall surrounding the anterior end of the tooth which Thorne (1930) did not recognise as a guiding ring in *Nygolaimus* and which he figured without comment in *Sectonema*. T. Goodey (1951) said that *Sectonema* had no guiding ring. Thorne's (1930) figure 7a of *N. brachyurus* (Fig. 2D) shows a wavy line, about half a head-width from the anterior end, across the stoma (the plicated anterior fixed ring) and then a thickish-walled part of the stoma divided into three sections. On one side of the figure this thick-walled part is separate from the continuation of the stoma wall below the fixed ring, and seems to be the guiding sheath, but usually the guiding sheath and stoma wall are adpressed closely together so that their separate nature is obscured. Thorne (1930) (repeated in Thorne, 1939 and T. Goodey, 1951), figured the way in which the spear of *N. aquaticus* is brought forward, accompanied by an evagination of the thick-walled guiding sheath (Figs. 2F, G and H). In this species the guiding sheath is in two sections only and, as evagination occurs, the first section curves inwards and forwards and becomes clearly separate from the stoma wall. The lower end of the first section appears to be joined outwardly to the stoma wall—the same appears so for *N. brachyurus*—as already described above for other Dorylaimids. The second section of the guiding sheath appears to be fused, at the level of the base of the mural tooth, with the stoma wall and corresponds to the lower "inward" junction of Dorylaims described earlier. When the tooth is completely evaginated (Fig. 2H) this basal attachment is quite clear.

Thus the nature of the guiding apparatus in Nygolaims appears to be essentially similar to that described earlier for other Dorylaimids and this common pattern probably exists throughout the Dorylaimidae.

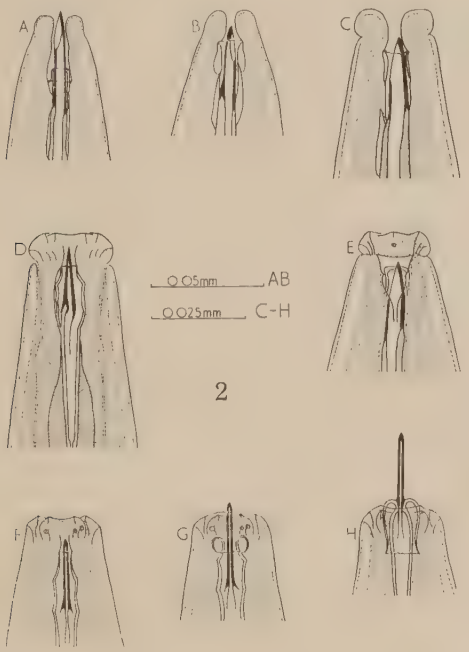


Fig. 2.—A. *Dorylaimus stagnalis*—relationships of guiding sheath to stoma wall and spear, showing the three rings of fusion and the sheath well evaginated beyond anterior fixed ring (original). B. *Aporcelaimus minor*—showing similar relationships but no evagination of the sheath (original). C & E. *Aporcelaimus obscurus*—C. (original) present interpretation of relationships between guiding sheath, stoma and spear; E. (after Thorne & Swanger, 1936, Fig. 151) for comparison; D. *Nygolaimus brachyurus*—to show guiding sheath separate from stoma wall but with similar relationships of sheath to stoma and spear as in other Dorylaimoids (after Thorne, 1930); F, G & H. *Nygolaimus aquaticus* three positions of the spear showing the progressive eversion of the guiding sheath (after Thorne, 1930).

Pairs of photographs taken at different levels of focus show, for *Dorylaimus stagnalis* and *Aporcelaimus minor*, the nature and relationships of the guiding sheath to the stoma and spear (Plate I AB and CD respectively).

The position of *Dorylaimus obscurus* Thorne and Swanger, 1936 in the genus *Dorylaimus* is questionable. Specimens collected by Thorne and T. Goodey in Utah in 1947 and named at that time show a funnel-shaped anterior fixed ring that is sometimes plicated, which suggests that the specimens are Aporcelaims. Plate 1E is a photograph of a moulting specimen showing the funnel-shaped nature of the end of the guiding sheath. Fig. 2C shows the nature of the guiding apparatus in a specimen with a plicated fixed ring and Fig. 2E is a copy of Fig. 151 of Thorne and Swanger for comparison. On this basis, *D. obscurus* is renamed *Aporcelaimus obscurus* (Thorne and Swanger, 1936) n.comb.

References

Thorne, G. and Swanger, H. H., 1936.—"A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n.g. *Dorylaimoides* n.g. and *Pungentus* n.g." *Capita zool.*, **4**, 1–223. (W.L. 5943).

Thorne, G., 1939.—"A monograph of the nematodes of the superfamily Dorylaimoidea." *Ibidem*, **8**, 1–261.

Goodey, T., 1951.—Soil and freshwater nematodes. London, Methuen, 390 pp.

Williams, J. R., 1959.—"Studies on the nematode soil fauna of sugar cane fields in Mauritius. 3. Dorylaimidae (Dorylaimoidea, Enoplida)." *Occas. pap.* (3), *Mauritius Sug. Industr. Res. Inst.* 28 pp.

Thorne, G., 1930.—"Predacious nemas of the genus *Nygolaimus* and a new genus, *Sectonema*." *J. agric. Res.*, **41**, 445–466. (W.L. 10966).

Goodey, J. B., Peacock, F. C. and Pitcher, R. S., 1960.—"A redescription of *Xiphinema diversicaudatum* (Micoletzky, 1923 and 1927) Thorne, 1939 and observations on its larval stages." *Nematologica*, **5**, 127–135.

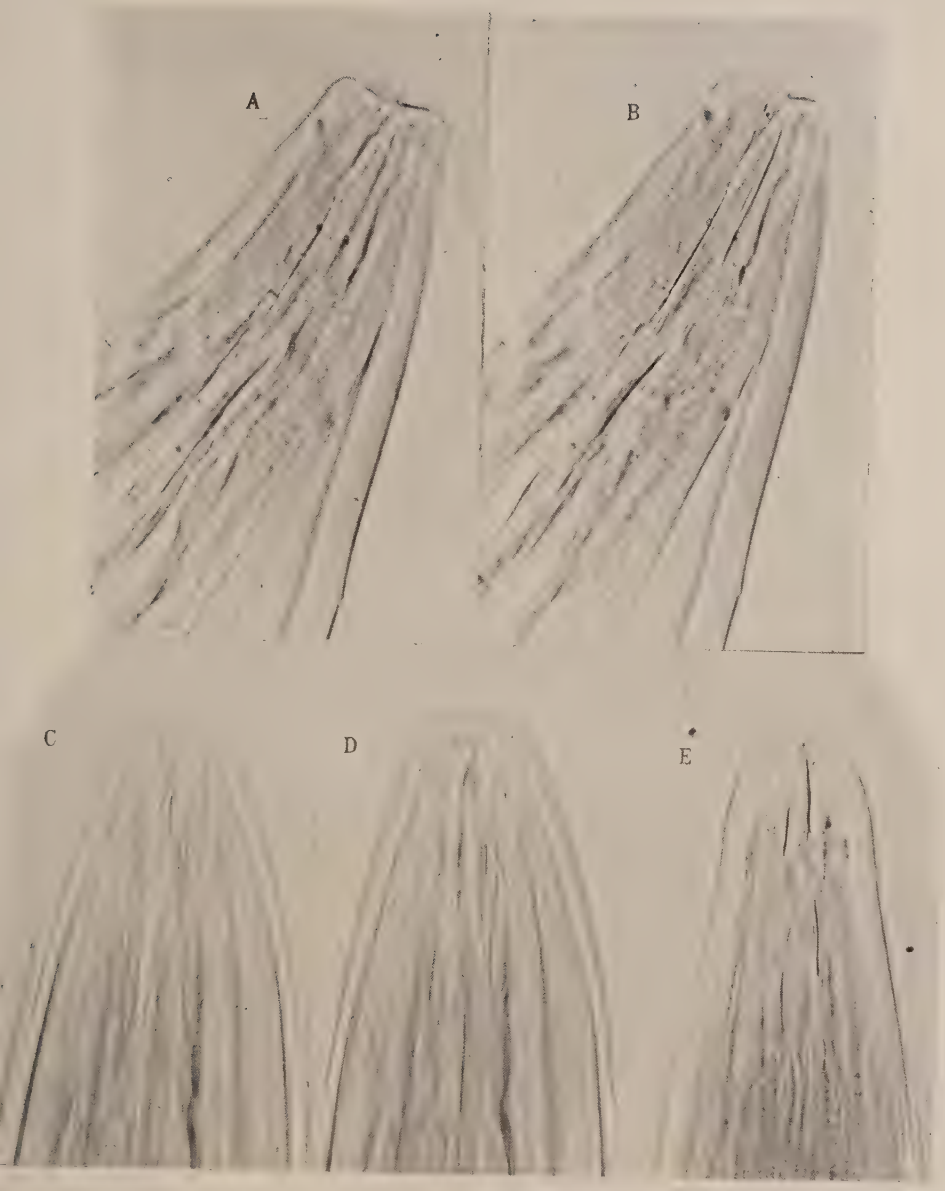


PLATE I. Microphotographs showing the relationships of guiding sheath to stoma wall and spear;

A & B for *Dorylaimus stagnalis* (c.f. Fig. 2A); C & D for *Aporcelaimus minor* (c.f. Fig. 2B); E. *Aporcelaimus obscurus* moulting specimen (c.f. Fig. 2 C & E). (A & B, C & D are at levels 4μ apart).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 107–116.

Passage of Eggs by Hosts Infected with *Schistosoma haematobium**

By Robert E. Kuntz**

Parasitology Department, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan (Formosa) and NAMRU-3, Cairo, Egypt (UAR)

Various relationships including the pathogenicity of the parasite to man or other mammals and the ability of a given animal to serve as a satisfactory laboratory host or possibly as a reservoir are dependent upon the egg laying capacity of the schistosomes within their definitive hosts. Since a number of small animals were infected during the course of susceptibility studies relating to the biology of *Schistosoma mansoni* and *S. haematobium* in Egypt (Kuntz, 1955) there has been ample opportunity to follow the general egg laying pattern of the latter species. Consideration is given to this aspect of the biology of this parasite since the egg laying ability of the schistosome within the host is associated with susceptibility and is related to host-parasite compatibility. Furthermore it has been the assumption of some investigators that *S. haematobium* is too poorly established in small mammals to produce viable eggs which may be passed by the host.

The present report discusses the egg production by *S. haematobium* in laboratory and wild mammals available at U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Material and Methods

Although emphasis was given to the smaller mammals (mostly rodents) which had been shown to be moderately susceptible to infection by *S. haematobium* (Kuntz and Malakatis, 1955a, 1955b) some of the less susceptible animals were also used.

Hosts employed were :

Mus musculus praetextus Brants—the common house mouse of Egypt.

Rattus rattus (L.)—the common house rat.

* The opinions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department.

** Present mailing address : Box 14, APO 63, San Francisco, California.

Arvicanthus niloticus (Desmarest)—the Nile rat, common to the irrigated and cultivated areas of Egypt.
Acomys cahirinus (Desmarest)—the spiny back mouse.
Gerbillus pyramidum Geoffrey—the gerbil or common desert mouse.
Meriones s. shawi Duvernoy—the jird, a desert rodent.
Nesokia indica suilla Thomas—the Egyptian bandicoot, found in the cultivated areas.
Hemiechinus auritus aegyptius Fischer—the Egyptian hedgehog (insectivore).
Papio hamadryas—baboon.
Cercopithecus—monkey.

Domestic mammals included albino mice and rats, and golden hamsters.

All hosts were exposed to 200 cercariae of *S. haematobium* obtained from naturally infected *Bulinus* which had been dipped from the irrigation systems near Cairo. Cercariae were pooled from 10 to 20 snails prior to exposure of hosts. Hosts were exposed to cercariae which emerged from the same stock of infected snails. All animals were partly immersed in warm water for 15 minutes prior to exposure to encourage passage of urine and faeces which, if present, might alter the infective potential of the cercariae. The smaller mammals were exposed in a cercarial suspension in $\frac{1}{2}$ inch of water in 500 ml. crystallizing dishes, whereas baboons and monkeys were held securely in larger glass containers.

Examination of faeces was begun at 30 to 35 days after exposure to cercariae and continued at 2 to 4 days intervals for a period of 4 to 6 weeks. Thereafter pellets were examined at weekly intervals until the host was sacrificed or died. In the beginning an attempt was made to examine faeces and urine separately. This proved impractical however and emphasis was given to examination of faeces after the initial series of hosts in susceptibility studies (Kuntz and Malakatis, 1955a, 1955b) had passed so few eggs with the urine.

An accumulation of faeces for a 24 hr. period was used for egg determinations. Pellets were collected from the bottom of mouse jars in which small mammals were maintained and from catch pans under the cages of the monkeys and baboons. After maceration and mixing in normal saline, faeces were strained through 60 mesh screen wire to remove the coarse debris; then were allowed to stand for

5 to 10 minutes between each of 3 washings. Sediment from final wash was examined on microslide by low magnification of a compound microscope. One quarter of the sediment was examined. The total number of eggs was calculated by multiplying the number of viable and nonviable eggs by 4.

After several weeks with a negative record for passage of eggs some of the hosts were sacrificed. Hosts were examined at this time or at time of death to obtain the adult schistosomes.

RESULTS AND DISCUSSION

Approximately 120 mammals were exposed to infection to insure an adequate number of hosts for study of egg passage in the different species of animals represented. The original number for each species was reduced considerably since some animals escaped, some were injured in handling and others died due to infection during the period of study. Since there was some deviation in the intervals between time of exposure and the appearance of the first eggs, in the schedule of faecal examinations, in the duration of infection before the host died, etc., the data cannot be satisfactorily grouped or combined for each host species. Therefore in Table I pertinent data relating to egg passage are given for individual hosts.

Albino and wild rats (*Rattus rattus*) are not listed in the table since the infection in the former was entirely self terminating and only a few poorly developed worms occurred in the latter. Neither host passed eggs and only a few scattered nonviable eggs were found in the liver of wild rats at time of autopsy. Although the hedgehog (*Hemiechinus*) was shown previously to be a fair host for *S. haematobium* only 1 of 3 surviving hosts passed a few nonviable eggs during the course of this investigation.

Data obtained from similar observations on baboons, monkeys and several children, for the purpose of comparison with findings in lower vertebrates, are not given in Table 1 since egg passage records were limited and were made at irregular intervals.

Previous studies of the susceptibility of laboratory and wild mammals indicated the great variation in parasite recoveries and a wide range of pathologic conditions accompanying experimental infections with *S. haematobium*. Variations in egg passage by different

host species as well as by individuals were of a comparable magnitude. The passage of eggs was erratic in most of the animals studied and the interval between infection and the first occurrence of eggs in the faeces ranged from 7 to 18 weeks, depending upon the individual host. As a rule only limited numbers of eggs (4 to 10 up to 16 or 20/day) were found during the first few days that eggs were detected. In some hosts egg counts jumped to 200/day at the end of the first week of passage, followed by a period of 8 to 10 days when the records were negative. Egg passage however was more consistent in hosts (*Mus musculus* and *Nesokia indica*) in which the overall egg counts were the greatest. One of the 7 bandicoots studied (NI–3) passed both viable and nonviable eggs on every examination made over a period of 4½ months and most of these hosts produced eggs until sacrificed.

In one albino mouse (AM–4) moderate numbers of eggs occurred in pellets regularly for more than 35 weeks. The egg output was reduced markedly from the 60th to the 80th weeks at which time the mouse died. Although no parasites were present at autopsy a few eggs, the majority nonviable, were found in the walls of the small intestine. Nonviable eggs were passed by this host only 2 weeks prior to death, indicating that eggs trapped in the intestinal wall were released for some time after natural termination of the infection. A similar relationship occurred in 3 of 4 *Gerbillus*.

Although the viability during some examinations was determined by hatching, in most cases viability was judged by general appearance of the eggs. In experimental bilharziasis there has been a tendency by some investigators to judge host-parasite compatibility by the number of viable eggs passed by the host. Studies in our laboratory indicate that this is only one of several criteria to be employed. Both man (presumably the ideal host) and lower vertebrates pass viable and nonviable eggs of *S. haematobium* in varying ratios, depending, perhaps, on the status of the infection in the individual. Release of eggs into the intestinal tract or into the urinary bladder occurs as a result of a pathologic phenomenon at a given site. A study of egg deposits in lower vertebrates suggests that the viability of eggs when passed, is related, in part, to the duration of their residence in host tissues. When a lesion for the escape of eggs develops in an area where eggs have been recently deposited it seems likely that a greater number of eggs will be viable. On the other hand, eruption of eggs which have been trapped for some time may account for a greater proportion of nonviable eggs. With such factors at play a wide range of viability figures (0–100 per cent) (Table 1) is to be expected and it

TABLE I

Eggs of SCHISTOSOMA HAEMATOBIUM *passed with faeces of infected rodents*

Host	Duration of egg production (weeks)*	Peak of egg production (weeks)*	Percentage of eggs viable	Number of eggs per day**	Worms present at post-mortem examination			
					Males	Females	Pairs	Total
Albino mice								
AM–1***	21	2–3	32	115	1	2	13	29
AM–2***	78	21–27	28	14	1	0	0	1
AM–3***	41	6–8	24	19	—	—	1	2
AM–4	77	5–8	56	38	—	—	—	0
AM–5***	18	4–6	18	41	2	4	4	14
House Mice—*Mus musculus*								
MM–1***	13	12–15	14	111	2	—	4	10
MM–2	56	18–29	36	96	4	1	2	9
MM–3	56	6–8	53	58	9	—	5	19
MM–4***	13	1–2	27	151	—	4	5	14
MM–5	14	2–3	71	14	2	—	2	6
Golden hamsters								
H–1	36	1–2	0	4	0	0	0	0
H–2***	32	25–27	50	4	2	0	0	2
H–3	44	13–15	95	16	4	0	2	8
H–4	28	6–18	57	114	4	0	2	8
Nile Rats—*Arvicanthis niloticus*								
AN–1	41	11–15	12	51	0	0	6	12
AN–2	147	15–23	43	18	2	0	1	4
AN–3***	83	10–16	23	19	1	2	0	3
AN–4	98	11–13	16	16	0	0	10	20
Spiny Back Mice—*Acomys cahirinus*								
AC–1***	24	6–7	50	8	2	0	3	8
AC–2	16	9–11	10	3	1	3	0	4
Gerbils—*Gerbillus pyramidum*								
GP–1	21	6–8	31	99	0	0	4	8
GP–2	29	10–13	30	21	0	0	0	0
GP–3***	37	14–17	27	6	0	0	0	0
GP–4	81	7–22	26	98	0	0	0	0
Jirds—*Meriones shawi*								
MS–1	16	9–10	0	6	2	0	1	4
MS–2	34	26–28	94	4	0	0	1	2
MS–3***	57	8–11	83	12	6	3	1	11
MS–4	32	25–28	100	2	0	0	1	2
Bandicoots—*Nesokia indica*								
NI–1	24	7–10	24	109	1	0	3	7
NI–2***	21	9–11	9	181	0	0	10	20
NI–3***	19	9–12	4	354	0	0	19	38
NI–4***	33	11–16	34	76	0	0	10	20
NI–5	38	6–11	34	91	5	7	30	72
NI–6***	52	6–7	79	15	5	2	3	13
NI–7	17	6–8	28	157	0	0	14	28

* weeks after first detection of eggs in faeces.

** eggs/day (mean) computed as total number of eggs/number of examinations.

*** host died due to infection, old age, etc.

is not believed that a low percentage of viable eggs is necessarily an accurate expression of compatibility between the schistosome and its host.

In the case of human bilharziasis it has been shown that vigorous exercise tends to encourage the passage of greater numbers of eggs of *S. haematobium* with urine. Similar relationships have been observed for the egg counts in faeces from *Arvicanthis*. During the peak period of egg passage for *Arvicanthis* which by previous susceptibility studies had been considered an ideal host, egg counts were made for pellets passed during hours of darkness for comparison with those passed during the day. The egg passing activities for 3 bandicoots were followed daily for 2 weeks. The number of pellets and eggs passed in the early morning (0500 to 0800) were considerably greater than those passed in the late afternoon, the increase being in the range of 15 to 25 per cent. These observations, although limited, probably are to be expected since the nocturnal bandicoot is extremely active during the hours of darkness. This, coupled with an increase of intestinal activity as a result of greater food consumption probably facilitates discharge of eggs from lesions into the lumen of the intestine. It is doubtful that the same results would have been obtained in *Arvicanthis* subsequent to the period of most active egg passage when there would have been less tendency for tissue trapped eggs to escape from older lesions.

During the course of infection in rodents the rates of egg passage had a tendency to follow a similar pattern in most individuals, regardless of the number of eggs passed with stools. The pattern was one of a normal curve with counts low for the first week to 10 days, followed by a moderate to marked increase over a period of one to several weeks. After the peak was passed the rates were erratic or fluctuating but gradually decreased. It should be noted however that in earlier susceptibility studies (Kuntz and Malakatis, 1955b) that 2 of 11 *Arvicanthis* passed moderate numbers of viable and nonviable eggs for approximately 3 years. There is no apparent explanation for the great variation in the times at which the peak of egg passage was attained. In some hosts [albino mouse (AM–2) ; hamster (H–2) ; gerbil (GP–3) ; jirds (MS–2 and 4)] the peak was not realized until the infection was nearing termination. The latter seems to indicate some type of alteration in the host which permits the escape of eggs through the intestinal wall at a late date. An examination of hosts which had ceased to pass eggs for several weeks showed that there were ample quantities of eggs in the walls of small and/or large intestine to suggest the finding of eggs in the faeces. However, there

were no haemorrhages or active sites of inflammation which would have permitted normal escape of eggs into the intestinal lumen.

The location of egg laying parasites and the site of egg deposition are of primary concern if the schistosomes are to realize the most satisfactory relationship for continuation of the life cycle. Undoubtedly, the location of schistosomes in the host is governed, in part, by chance, and even in man (Alves, 1958) *S. haematobium* may not necessarily attain access to a habitat from which eggs may be deposited in the urinary bladder. Although fairly accurate observations can be made in experimentally infected animals there was no clear correlation between the sites of egg deposition and the numbers of eggs passed with faeces. Postmortem examinations revealed that eggs in some hosts were restricted to the small intestine, whereas in others they were localized in the caecum or in the lower large intestine. Counts, however, were low or negative for individual hosts in which adult schistosomes were found only in the liver, suggesting their residence there for some time. Residence of schistosomes in the liver or in a position where eggs are transferred to this organ represent fruitless productions in which egg passage in faeces is effected regardless of the fecundity of the parasite. At this time it is unknown whether severe host reaction to large deposits of eggs in one organ, such as the liver, influences the host's response to eggs and their ultimate release from other locations in the body.

As indicated in Table I the number of eggs passed by an infected host, in general, was related to the number of schistosomes present. However there were exceptions in which only limited numbers of eggs were passed by the host in spite of the presence of several pairs of schistosomes, the females with numerous eggs in the uterus. For some hosts with moderate numbers of worms the egg counts dropped to near nil and remained as such for weeks. Upon autopsy after death several mammals possessed few or no eggs in the intestinal walls although the adult schistosomes were in a position that would seem to permit easy access to vessels associated with the intestinal tract. Myriads of eggs were present in the liver. In such instances low counts or an absence of eggs in faeces seem to indicate unsatisfactory physiological relationships as well as a shortcoming relating to ideal position in the host. Possibly these are expressions of incompatibility resulting from insufficient nutritional requirements and a lack of accommodation in small blood vessels in diminutive hosts.

Passage of eggs in the urine and faeces of baboons and monkeys infected with 200 cercariae of *S. haematobium* was followed for short

periods to permit an estimate of egg output and their fluctuation in larger hosts. Eggs were detected in faeces and urine of primates at approximately the same time as found in faeces of rodents. During one month of observation on 3 baboons and 3 monkeys the egg counts and viability ratios were erratic but similar to that recorded for the rodents. Twenty four hour stool specimens from monkeys yielded from 15 to 55 eggs/day and on several occasions reached 400 to 600/day. The counts for baboons studied concurrently were slightly greater. On some occasions the number of eggs was considerably greater than listed for the rodents but the overall output based upon the number of eggs to the number of schistosomes recovered at autopsy fell in the same general range. These data are in contrast with the information of Pesigan *et al.* (1958) who observed the passage of eggs by wild rats infected with *Schistosoma japonicum* in the Philippines to be only a fraction of the mean daily egg output by larger vertebrates.

Limited observations were made also on total egg output in the urines of 4 infected children (9 to 13 years of age) from an endemic area near Cairo. Over a period of 4 weeks egg counts ranged from 16 to 7745/day and the percentage of viables from 68 to 87 per cent. These data point to a similarity of egg production activities in man and lower vertebrates but obviously cannot be used for critical comparison since the number of parasites and status of infection in the former are unknown.

These cursory observations on egg passage by lower primates and man suggest patterns of egg production similar to those characteristic of some of the more suitable lower mammals. Apparently passage of eggs by *S. haematobium* is an activity with great ranges in variability whether in large or small hosts. Barlow (1948) has suggested that eggs of this schistosome in man are passed in constant numbers daily. On the other hand Gerritsen *et al.* (1953) have pointed to the enormous variation of eggs found in human urines and have warned of the unreliability of diagnosis of bilharziasis on single samples. Possibly negative findings or a paucity of eggs from experimentally infected hosts have been due, in part, to limited faecal samplings. Data presented herein also indicate that egg counts in faeces of lower vertebrates cannot be used for accurate estimation of intensity of infection. The same applies to human infections and Scott (1957) has emphasized that quantitative studies with *S. haematobium* to show correlation with intensity of infection must be based upon rigidly controlled conditions over a longer period of time than usually employed.

Until recently information relating to the susceptibility patterns in lower mammals infected with *S. haematobium* have been rather vague. In most, the trend of thought has been slanted toward the inability of this parasite to establish itself parasitologically in non-primate hosts. Consequently there have been few data revealing the egg laying potential of *S. haematobium* and egg production and deposition have been matters of conjecture. Moore and Meleney (1954) working with laboratory animals have given general information on the passage of eggs by *S. haematobium* in albino mice and hamsters, and later Moore and Sandground (1956) calculated the egg depositing capacity of *S. mansoni* and *S. japonicum*. These and other reports however, have not provided data on the actual numbers of eggs produced from small animals. It is believed that the present study fills a small gap in our understanding of experimental bilharziasis and points to the possibility, but not necessarily the probability, that some lower vertebrates may serve as reservoirs should proper circumstances permit.

SUMMARY

Study of a series of lower vertebrates and 2 primates infected with *Schistosoma haematobium* has revealed their egg producing or egg passing potentials. Viable eggs were not passed by albino or wild rats (*R. rattus*), or by hedgehogs (*Hemiechinus*). In hosts passing eggs the counts were not great but data do suggest host-parasite relationships which permit continuance of the parasite cycle. Egg passage was erratic and tended to follow a normal curve, i.e., few eggs at first, building up to a peak period after which the counts usually diminished. There was no close correlation between the sites of egg deposition along the intestinal tract and the numbers of egg produced. The peak of egg production was irregular in time after first egg detection as well as in duration. There was wide range in the percentages of viable eggs passed by different hosts and by individuals of the same species. Data reveal the possibility of lower vertebrates serving as reservoirs should satisfactory circumstances allow.

ACKNOWLEDGMENTS

The author is indebted to George M. Malakatis HM1, USN and to Said Kassim Mohamed of NAMRU-3, Cairo, Egypt for technical assistance.

References

Alves, W., 1958.—"The distribution of *Schistosoma* eggs in human tissues." *Bull. World Hlth. Org.*, **18**, 1092–1097. (W.L. 5748c).

Barlow, C. H., 1948.—"Une théorie sur la ponte de *Bilharzia haematobia*." *Ann. Parasit. hum. comp.*, **23**, 301–304. (W.L. 899a).

Gerritsen, T., Walker, A. R. P., de Meillon, B. and Yeo, R. M., 1953.—"Long term investigation of blood loss and egg load in urinary schistosomiasis in the adult African Bantu." *Trans. R. Soc. trop. Med. Hyg.*, **47**, 134–140. (W.L. 21671).

Kuntz, R. E., 1955.—"Biology of the schistosome complexes." *Am. J. Trop. med. Hyg.*, **4**, 383–413.

————, and Malakatis, G. M., 1955a.—"Susceptibility studies in schistoso miasis, III. Infection of various experimental hosts with *Schistosoma haematobium* in Egypt." *Exptl. Parasit.*, **4**, 1–20.

————, 1955b.—"Susceptibility studies in schistosomiasis. IV. Susceptibility of wild mammals to infection by *Schistosoma haematobium* in Egypt." *J. Parasit.*, **41**, 467–475. (W.L. 11428).

Moore, D. V. and Meleney, H. E., 1954.—"Comparative susceptibility of common laboratory animals to experimental infection with *Schistosoma haematobium*." *Ibidem*, **40**, 392–397.

————, and Sandground, J. H., 1958.—"The relative egg producing capacity of *Schistosoma mansoni* and *Schistosoma japonicum*." *Am. J. Trop. med. Hyg.*, **5**, 831–840.

Pesigan, T. P., Farooq, M., Hairston, N. G., Jauregui, J. J., Garcia, E. G., Santos, A. T., Santos, B. C. and Besa, A. A., 1958.—" Studies on *Schistosoma japonicum* infection in the Philippines. 1. General considerations and epidemiology." *Bull. World Hlth. Org.*, **18**, 345–455. (W.L. 5748c).

Scott, J. A., 1957.—"Egg counts as estimates of intensity of infection with *Schistosoma haematobium*." *Texas Rep. biol. Med.*, **15**, 425–430. (W.L. 21126a).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 117–126.

Some Problems in Bilharziasis in Africa and the Adjoining Countries

By P. L. LeRoux
Department of Parasitology, London School of Hygiene and Tropical Medicine

As an old student of Emeritus Professor R. T. Leiper it gives me great pleasure to contribute something for the Anniversary number. It was indeed an ordeal for me to decide what to contribute in honour of this famous man who is happily still with us.

He has contributed much, by training and encouraging helminthologists, to alleviate pain, miseries, losses and mortality from helminthiases in man and man's domesticated animals. As Director of the Commonwealth Bureau of Parasitology he rendered a great service not only to the British Commonwealth but also to the rest of mankind in the world. He is still very much interested in helminthology. Like Cecil Rhodes, the founder of Rhodesia, he undoubtedly also feels : "So much to do and so little done."

Many of his students will recall how they were started off on that venture "Helminthology" at the Albert Docks, at Ensleigh Gardens and later at Keppel Street. They had to make *camera lucida* drawings of mounted stained preparations of trematode and cestode parasites of man and animals to begin with and later of nematodes. Every now and again he had a look at the drawings with critical but kindly eyes and with a fatherly smile suggested that the artist go back and have another look at certain structures in the preparations or specimens. It was by looking and looking again that the student saw what should be seen and developed the love for looking to see more and more.

As Professor Leiper is still much interested in bilharziasis I will venture to guess at what would be included in his reply if he were asked what subjects in helminthology need further investigation. There would probably be words to the effect : "Go back and have another look at bilharziasis."

Some of the problems associated with the various members of the family Schistosomatidae parasitizing man, his domesticated

animals, wild mammals and birds, which need re-investigation are briefly these :—

(a) The validity and the geographical distribution of the various species of schistosomatids described from man and mammals in Africa and the adjoining countries.

(b) The validity and the geographical distribution of the various species of fresh water molluscs recorded as the intermediate hosts of schishosomatids parasitizing man and animals in Africa, Europe and the near East.

(c) The pathogenesis and pathology associated with invasions of the human and animal bodies by the various species.

(d) Immunity in schistosomatidosis.

(e) The control and eradication of bilharziasis.

The validity and geographical distribution of the various species.

Of the twenty-one species of schistosomatids which have been reported from man and mammals in Africa, Europe and the Near East only ten or eleven may be valid species.

The species *Schistosoma magna* Cobbold, 1859 and *S. faradjei* Walkiers, 1928 are so imperfectly described that they should be ignored ; *S. spindale* Montgomery, 1906 recorded by LeRoux (1933) from equines, cattle, sheep and several antelopes in Northern Rhodesia has proved to be a new species and named *Schistosoma leiperi* LeRoux, 1955. *S. spindalis* var. *africana* Porter, 1926 appears from the description of the species by Porter (1938) to be *Schistosoma mattheei* Vegalia and LeRoux 1929 ; the description of *S. curassoni* Brumpt, 1931 is based on scanty material and it seems probable that it may be either *S. bovis* Sonsino, 1876 or *S. mattheei*. Material obtained from the Veterinary Research Laboratory at Brazzaville and from the Veterinary Laboratory at Fort Lamy contains females with *in utero* eggs resembling those of *S. bovis* and eggs resembling those of *S. haematobium*. There were also eggs which are intermediate in outline between those of *S. bovis* from the Sudan, Spain, Iraq and Sardinia and *S. mattheei* from Southern Africa (Northern Rhodesia, Southern Rhodesia, Bechuanaland, Transvaal, Portuguese East Africa, Cape Province and Natal). Very abnormally shaped eggs have been encountered in specimens of *S. mattheei* collected from cattle aged between 15 to 20 years old in Northern Rhodesia. The identification of *S. indicum* from a zebra in N. Rhodesia by LeRoux (1933)

was based on eggs observed in females collected from an old zebra mare and the writer takes this opportunity to observe that a re-examination of the specimens suggests that the eggs were abnormal and that *S. indicum* should no longer be considered as occurring in Africa. Microphotographs of schistosomes recovered by Becker (1916) from a guineapig exposed to "wild" cercariae from "wild" specimens of *Bulinus* (*Physopsis*) *africana* in the Transvaal suggest that the species involved was *S. mattheei* and not *S. haematobium*. It is doubtful whether the species *S. bovis* occurs in Southern Africa. The records of *S. japonicum* from indigenous cases in South Africa and in Tanganyika are based on eggs encountered in human faeces. In the Tanganyika cases the presence of the eggs in the human stools may have followed the ingestion of organs of antelopes infected with *Rhodobilharzia margrebowiei* (LeRoux, 1933) LeRoux, 1958.

The differentiation between *S. haematobium* (Bilharz, 1852) and *S. capense* (Harley, 1864) LeRoux, 1958, is based on the shape of the eggs and the much more posterior situation of the ovary in the first mentioned species. The generally accepted description of the females of *S. haematobium* is based on material recovered from experimentally infected monkeys and not on material recovered from human beings.

In Bilharz's original description of the female recovered from the portal vein the ovary is figured as situated about the junction of the anterior two thirds and the posterior third of the female. The fact that the specimen was recovered from the portal vein suggests that it may have been an underdeveloped specimen. In experimental animals such as mice, hamsters and monkeys the ovary in young specimens of *S. capensis* is often further back than in older specimens. This has also been observed in specimens of *S. bovis*, *S. mattheei*, *S. intercalatum* and *S. leiperi* from laboratory animals. Attempts should be made to collect specimens of *S. haematobium* from man in Egypt, Iraq, Iran, South Africa and elsewhere so that the morphology of the specimens from these different areas can be compared as regards the location of the ovary in the specimens from these areas. The examination of recently received collections of eggs from cases of urinary bilharziasis in Iraq, Iran and Nigeria showed the presence of many somewhat elongated to almost spindle-shaped eggs. These eggs were more elongated than those of *S. capensis* from South Africa and could only be differentiated from *S. mattheei* by the nature of their terminal spines which are not as stout as in *S. mattheei*, *S. bovis* and *B. intercalatum*. Eggs of *S. capensis* type were also present in the collections from Nigeria, Egypt, Iraq and Iran. The eggs showed great pleomorphism. The material from Nigeria was forwarded here for identification as the sender believed that more than one species may be

involved. This variation in shape and size encountered in the eggs within a species suggests that the various species are still in an active process of evolution and that the evolution has progressed further in some areas than in others. Whether the interbreeding of species from man and from animals does occur is uncertain but it might account for this pleomorphism in shape and size of the eggs in some areas.

It should also be observed that little is known about the influence of zoologically widely separated host species on the normal physiological functions within a species that had been geographically isolated in one host for some time.

The shape and size of the eggs of a strain of a species vary in the common laboratory animals with the age of the infection. This variation is often greater at the commencement of egg production than later on. The variation has been observed to be considerable in cases where the experimental animals have been heavily exposed and the normal development of the worms had been retarded undoubtedly by overcrowding with resultant under nourishment. This break-down of normal development manifested by the production of abnormal eggs, can also be attributed to the period of time that the snails from which the cercariae had been obtained, had been infected ; and also to the vitality of the worms in subsequent hosts. There are various other environmental factors acting on the worm both in its intermediate host and in the definitive host, which seem to influence its normality as regards its normal morphology and its pathogenesis for the definitive host.

The shape and size of the egg in the different species is determined by the differences in the rate and degree of dilatation and contraction of the common duct formed by the union of the oviduct and the vitelline duct before entering the ootype either terminally, subterminally or laterally, according to the species. In those species, e.g. *Rhodobilharzia margrebowici*, and other species, in which the eggs are symmetrically oval to rounded with a minute lateral or terminal spine according to the species, the dilatation and contraction of the common duct is evidently very sudden. In *Schistosoma leiperi* in which there are prominent polar prolongations with the posterior prolongation terminating in a prominent spine, the dilatation of the common duct begins slowly and the anterior prolongation is formed ; it then dilates and contracts more sharply and the more dilated part of the egg containing the ovum and vitelline material is formed ; the contraction slows down and the posterior polar prolongation terminating in a prominent spine is formed. It seems definite that the spine is always prominent when the final contraction of the duct

is slow. In the case of *S. bovis* the spine is not infrequently heart-shaped as figured by Sonsino. In some eggs this heart-shaped spine is connected to the posterior end of the egg by a long thin "neck".

In the Asiatic mammalian schistosomes *S. spindale* and *S. incognitum* the eggs are not symmetrical in all planes and this suggests that only about threequarters of the circumference of the common duct dilates during the passage of the ovum and the accompanying vitelline material. In the case of *S. spindale* this passage is undoubtedly less rapid than in *S. incognitum* and hence the long polar prolongations and the large terminal spine in the first mentioned.

In *Eurobilharzia bomfordi* or *Afrobilharzia rodhaini* each of which has a very characteristic short anterior subterminal prolongation and a prominent stout subterminal spine, a short slow dilatation is followed by a sudden dilatation which terminates in a short slow contraction.

In *Schistosoma haematobium* with its oval to elongated, occasionally somewhat spindle-shaped eggs furnished with a relatively fine terminal spine of very variable length in experimental animals and even in man, it is undoubtedly the rate of dilatation and contraction of the common duct which influence the shape and size of the eggs. Specimens of urine from Egypt, Iraq, Iran and parts of West Africa showed greater pleomorphism in the shape and size of the eggs of this species from man than has been observed by me in eggs from human beings in Southern Africa where the closely related species *Schistosoma capense* occurs.

The identification of *Schistosoma* spp. with absolute certainty is well nigh an impossibility if the criteria employed are the shape and size of eggs recovered from urine or faeces.

Identification and geographical distribution of the intermediate hosts of schistosomatids parasitizing man in Africa, Europe and the Near East.

The identification of the African freshwater mulluscs which act as intermediate hosts of mammalian schistomes and other trematodes parasitizing man and animals leaves much to be desired. It is doubtful if there are really as many different species as some workers accept.

The publication of Leiper's discoveries in Egypt and the spread of bilharziasis in Africa and its discovery in man and animals in places in Europe and the Near East initiated investigations which revealed that it can now be accepted that most of the members of the molluscan sub-family Bulininae can act as intermediate hosts of the African

terminal-spined *Schistosoma* spp. In nature *S. haematobium* and *S. bovis* are transmitted essentially by *Bulinus* (*Bulinus*) *truncatus* in North Africa, parts of Africa south of the Sahara and in the Near East. In Europe *S. haematobium* is transmitted in Portugal by *Planorbarius metidjensis*. In Corsica, Sardinia and in Sicily *B.* (*B.*) *truncatus* acts as the intermediate host of *S. bovis* while in Spain the intermediary is evidently *P. metidjensis*. There is no proof as yet that the last mentioned mollusc acts as intermediate host of *S. haematobium* or *S. bovis* in those parts of North West Africa where it occurs.

In parts of Africa from south of the Sahara to well into the Congo, *S. haematobium* and *S. bovis* have *Bulinus* (*Bulinus*) spp. as intermediaries while *S. capense* and *S. intercalatum* are transmitted by *Bulinus* (*Physopsis*) spp. All the described species of this subgenus of *Bulinus* must be accepted as potential intermediaries. In Africa south of the Congo *Bulinus* (*Bulinus*) *forskalii* and *Bulinus* (*Physopsis*) *africanus* and *B.* (*P.*) *globosus*, especially the two last mentioned, seem to be the intermediate hosts of *S. capense, S. mattheei* and *S. leiperi*. The susceptibility of *B.* (*B.*) *senegalensis* and *B.* (*B.*) *forskalii* to experimental infections with *S. haematobium, S. capense, S. bovis, S. mattheei, S. intercalatum* and *S. leiperi* suggests that the members of the "senegalensis" group of bulinines may be more important intermediate hosts than are as yet accepted. They are also the intermediaries of *Gastrodiscus aegyptiacus*, a parasite of equine animals and of several paramphistomids of sheep and cattle. In Madagascar the intermediate hosts of *S. haematobium* are suspected to be *B.* (*B.*) *madagascariensis* and *B.* (*B.*) *mariei* according to Gaud (1955). Photographs of the shell of *B.* (*B.*) *liratus* from Madagascar published by Mandahl-Barth (1957) suggests that this species may be a member of the subgenus *Bulinus* (*Physopsis*) and hence a probable intermediary of *S. haematobium*.

In Mauritius the fresh water snail identified for me as *Bulinus* (*Bulinus*) *cernicus* has been found infected with a mammalian schistosome cercaria and with the cercariae of two paramphistomids parasitizing cattle in Mauritus and in Africa.

Afrobilharzia mansoni, A. rodhaini and *A. mansoni* var. *rodentorum* (? synonym *A. mansoni*) have as their intermediaries members of the genus *Biomphalaria*. There seem to be no valid reasons for medical men and veterinarians to attempt differentiation between the various African biomphalarias as they are all susceptible to the same trematode. Recent experimental exposures by the writer, of a *Biomphalaria* species from the vicinity of Addis Ababa, where bilharziasis mansoni is not endemic, to a strain of *A. mansoni* from Tanganyika revealed the snails to be susceptible.

Certain workers have claimed to have found *Planorbarius metidjensis* susceptible, on experimental exposure, to *A. mansoni*.

The generally accepted statement that miracidia of a schistosome species are only attracted by molluscan species which can act as intermediate hosts of that species is completely inaccurate. Miracidia of *A. mansoni* are attracted by several species of snails including members of the genera *Bulinus* (*Bulinus*), *Bulinus* (*Physopsis*), *Lymnaea, Planorbis, Segmentina, Melania,* etc. and have been seen to attach themselves to the antennae and foot of members of these genera. Snails which had been exposed heavily failed to develop an infection. It seems probable that the miracidia may actually have penetrated the bodies of these snail but failed to develop normally as they would have done in a suitable host. Host specificity in helminths is evidently linked with a specific nutritional requirement.

The molluscan host of *Rhodobilharzia margrebowiei* remains to be discovered.

The assessment of the importance of schistosome infections in man and animals

The importance of schistosome infections in man and in the domesticated animals has not as yet been thoroughly assessed.

The assessment of the Medical and Public Health importance of bilharziasis was recently considered at Geneva by a scientific study group together with methods for conducting surveys in bilharziasis endemic areas to assess the importance of the disease.

In cattle, mortality has been attributed to schistosomiasis bovis but it is doubtful whether this mortality was actually due to the schistosome infection. It seems probable that the mortality was actually due to heavy parasitism by immature paramphistomes in the duodenum.

In the case of "snoring disease" in cattle in India, the granulomatous growths in the nasal passages provoked by an infection with *Schistosoma nasalis*, respiration is greatly impaired and the working efficiency of draught animals consequently reduced.

The use of sheep, goats and cattle as experimental animals for studying the pathogenesis of schistosome infections may elucidate some of the problems associated with bilharziasis in man.

Immunity in Bilharziasis

Although there are helminthologists who believe that there is an immunity provoked by an invasion by schistosome cercariae there are many medical officers who maintain that treatment is practically useless because the treated persons become re-infected immediately on re-exposure. There are no valid experimental data to prove that those treated can be re-infected immediately after treatment.

There are some experimental data which suggest that an exposure to infection does confer an immunity but nothing definite is known about the longevity of that immunity. Most of these immunity studies have been conducted unfortunately on abnormal hosts, laboratory animals, using the species *Afrobilharzia mansoni* from man.

For testing the development of an immunity it seems desirable to use a natural host and expose it to one of its parasites and to challenge the presence of an immunity by exposing the infected host to another species parasitizing it in nature. Animals that have been infected could be treated and re-exposed for the presence or absence of immunity. In some experiments animals should be exposed to the cercariae of human or bird schistosomatids and the presence or absence of an immunity ascertained by exposing them to one or more of the species normally parasitizing them.

There is circumstantial evidence which suggests that the immunity from a natural infection in animals may be of more than six months duration or even longer, and that the cercariae of species from animals may immunize man against species of human origin and *vice versa*. In Sardinia, Corsica and Sicily where schistosomiasis bovis occurs, human cases of bilharziasis haematobia have never been observed although *Bulinus* (*Bulinus*) *truncatus* the intermediate host of *S. bovis* in Corsica and Sardinia is highly susceptible to experimental infection with *S. haematobium* from Egypt. In Egypt where the two human schistosomes *S. haematobium* and *A. mansoni* are prevalent, cases of schistosomiasis in animals are practically unknown although *B.* (*B.*) *truncatus* from Egypt is highly susceptible to experimental infection with *S. bovis* from Kenya.

The absence of *S. haematobium* from certain areas of Africa where *A. mansoni* infections are common cannot be attributed to the absence of suitable intermediate hosts of the first mentioned.

The absence of bilharziasis of man in Pakistan and India, except for the small focus at a place south of Bombay, may possibly also be attributed to the prevalence of animal schistosomiasis.

Whether the cercariae of the avian schistosomatids or other dermatitis producing cercariae can immunise man and animals against the mammalian schistosomes also needs investigation.

The low worm loads in man and animals in some parts of Africa south of the Sahara desert suggest that there is no annual build up of the worm load. Other evidence against the gradual build up of infection is the presence in animals, sheep and cattle of unisexual infections with males where animal schistosomiasis is endemic. These unisexual infections in animals which have been exposed to invasion every year for several years suggest further that the immunity was not due to some antigen present in miracidia which had been killed within eggs arrested in the tissues.

The heaviest worm loads in man seem to occur in regions where the temperature of the snail infested waters is ideal for rapid development of the parasite in its intermediate host, for this results in the production of numerous cercariae of high vitality. Probably no further re-infection occurs during the natural life of the parasites in the human body as long as the once infected human being is immunized annually by re-exposure to mammalian schistosome cercariae.

Control and Eradication of Bilharziasis

Although the researches of Professor Leiper in Egypt during the First World War revealed that two species of schistosomes infected man there and that the two species were transmitted by species of snails belonging to different sub-families little real progress has been made towards the control and eradication of bilharziasis in the known endemic areas. The present indications are that the disease in man is spreading and increasing in severity especially in irrigation areas with high temperatures during the non-rainy season.

In many of the irrigation areas it is not the canals themselves but the storage reservoirs where water is stored for short periods or drains and permanent pools formed by the leakage of water from the canals which constitute the habitats of snails and are ideal environments for the development of the parasites in their intermediaries and the passage of the cercariae to man. Animals often have no regular access to these storage reservoirs or drains and the snails are exposed only to schistosome infections of human origin.

In both the Northern and the Southern Hemispheres, snails infected with human or animal species of schistosomes are usually encountered in the endemic areas from about the beginning of August to the onset of the rains. The infection rate in the snails seems to reach its maximum about October to November depending on the onset of the rains. Where two rainy seasons occur, infection in the snails is present for some weeks before the rains commence. The effect of floods is to swamp the infected pools and thus the risk of infection to man is greatly reduced or completely eliminated. In the case of irrigated areas flooding is controlled and infection in the snail in the canals, drains and storage reservoirs will persist until the water temperature drops below 19°-20° C. In some tropical regions in Africa the infection in the irrigation canals and drains may be present throughout the year.

The application of molluscocides to snail infested waters should be timed to destroy the snails before the water temperature has been over 20°C. for three to four weeks.

References

Becker, J. G., 1916.—"A preliminary note on an intermediate host of *Bilharzia haematobium* in the Transvaal together with a description of the cercariae with which the molluscs were infected." *Med. J. S. Afr.*, **11,** 156. (W.L.13037).

Gaud, J., 1955.—"Les Bilharzioses à Madagascar et aux Isles Mascareignes." *Bull. Org. Mond. Santé,* **13,** 259–288. (W.L.5748c).

LeRoux, P. L., 1933.—"A Preliminary Note on *Bilharzia margrebowiei,* a new parasite of ruminants and possibly of man in Northern Rhodesia." *J. Helminth.*, **11,** 57–62. (W.L.11224c).

————, 1955a.—*Bulinus* (*Bulinus*) *cernicus* (Morelet) as vector of paramphistomids and *Schistosoma haematobium* in Mauritius." *Trans. R. Soc. trop. Med. Hyg.*, **49,** 293. (W.L.21671).

————, 1955b.—"A new mammalian schistosome (*Schistosoma leiperi* sp. nov.) from herbivora in Northern Rhodesia." *Ibidem,* **49,** 293–294.

————, 1958.—"The validity of *Schistosoma capense* (Harley, 1864) amended as a species". *Ibidem,* **52,** 12–13. (W.L.21671).

Mandahl-Barth, G., 1957.—"Intermediate hosts of *Schistosoma.* African *Biomphalaria* and *Bulinus.*" *Bull. World Hlth. Org.*, **16,** 1103–11 and **17,** 1–65.

Porter, A., 1926.—"Notes on the structure and life-history of a variety of *Schistosoma spindalis* Montgomery observed in South Africa." *S. Afr. J. Sci.*, **23,** 661–666. (W.L.11338a).

————, 1938.—"The larval Trematoda found in certain South African Mollusca with special reference to schistosomiasis (Bilharziasis)." *Publ. S. Afr. Inst. med. Res.*, No. 42. (W.L.17416).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 127–130.

Studies on Parasites of the Grey Squirrel (*Sciurus carolinensis* Gmelin) from South Eastern England

I.—Helminth Parasites

By J. N. Oldham

*Department of Pathology, Royal Veterinary College, London, N.W.*1

The widespread distribution in Britain of the grey squirrel prompted an investigation of the parasite fauna of this rodent to find if it was capable of acting as a disseminator of parasites to other mammals or birds, particularly domesticated ones.

Material and Methods

The 100 carcases examined were obtained from the following localities : Berkshire, 6 from Basildon, 22 from Streatley-on-Thames ; Hertfordshire, 40 from Berkhamsted ; Kent, 16 from Sittingbourne ; Oxfordshire, 2 from Goring-on-Thames, 9 from Goring Heath, 4 from Henley ; Wiltshire, 1 from Colne. More carcases were obtained during the spring and autumn months than at other times. The numbers of animals received each month were : January 1, February 2, March 17, April 15, May 11, June 11, July 4, August 2, September 7, October 25, November 5, December 0.

Most of the animals had been shot, some had been trapped and a few killed on roads by motor vehicles. After examining the carcase for ectoparasites, it was opened and the entire alimentary tract removed. The contents of the stomach, small and large intestines were separately cleaned of colouring matter and light debris by repeatedly adding water to them, allowing the mixtures to sediment and decanting the supernatant fluid. When sufficiently cleaned, the stomach and gut contents were examined, portion by portion, under a binocular dissecting microscope and all worms removed for identification. The organs remaining in the carcase were also scrutinized for parasites.

Results

No trematodes were found but cestode material was recovered from three animals. A single *Cysticercus fasciolaris* was found encapsuled in the liver of one squirrel obtained from Streatley-on-Thames. In a second squirrel from the same locality there occurred an immature tapeworm the identity of which could not be determined. From a third animal, obtained from Henley, two immature tapeworms were recovered but it was also not possible to identify these specimens.

Two species of nematodes were recovered. A single male *Ascaris lumbricoides* Linn., 1758 was found in the small intestine of a squirrel sent from the Berkhamsted area of Hertfordshire. The other nematode, *Trichostrongylus retortaeformis* (Zeder, 1800) Looss, 1905, was recovered from 34 carcases. It occurred in small numbers on most occasions, ranging from 1 to 12 specimens in 30 of the squirrels. One carcase, however, yielded 22 specimens, whilst a second yielded 30, a third 102 and a fourth 126. All of the 121 male worms recovered were examined and checked for their identity and about one-third of the 274 female specimens were also examined critically. It is of interest to note that infested carcases came from widely separated localities and were not restricted to one particular area. Thus, 2 infested carcases came from Basildon, 9 from Streatley-on-Thames, 4 from Berkhamsted, 8 from Sittingbourne, 7 from Goring Heath, 3 from Henley and one from Colne.

Discussion

Examination of the carcases has shown that *Trichostrongylus retortaeformis* is relatively wide-spread amongst grey squirrels in south eastern England. This nematode is well known as a common parasite of rabbits and hares in the United Kingdom and Sloan (1951), discussing its host distribution, mentions that, until 1937, it had been recorded from these rodent hosts and no others. He had, however, overlooked a paper by Cameron and Parnell (1933), published four years earlier, in which these authors reported the occurrence of *T. retortaeformis* not only in the blue or mountain hare, *Lepus timidus* (or *L. variabilis*), the European or brown hare, *Lepus europaeus*, and the rabbit, *Oryctolagus cuniculus* but also in the European or red squirrel, *Sciurus vulgaris*, and the grey squirrel, *Sciurus carolinensis*. In a private communication to Sloan, Leiper, J. W. G. stated that he had (unpublished) records of the occurrence of *T. retortaeformis* in

the grey squirrel and in the hedgehog, *Erinaceus europaeus*. Furthermore, Leiper (1937) found this parasite at examination *post mortem* of two goats which had grazed rabbit infested paddocks at Winches Farm, St. Albans, Hertfordshire. Rabbits in the area were found to carry comparatively heavy infections of *T. retortaeformis*, *Graphidium strigosum* and *Passalurus ambiguus*. Leiper was also able to obtain experimental verification of the susceptibility of goats to infestation by this parasite. He was under the impression that his findings constituted the first record of the natural occurrence in the goat of *T. retortaeformis*, and this is probably true so far as the United Kingdom is concerned, but a comment by Nagaty (1932) is worthy of note. Nagaty gives a morphological description of *T. retortaeformis* and mentions that its hosts are *Oryctolagus cuniculus*, *Lepus europaeus* and *Viscacia viscacia* and then adds the comment "Reported by Gedoelst, 1911 in goat, ox, sheep but this lacks confirmation." It would therefore appear that Leiper's finding confirms the report by Gedoelst in so far as the goat is concerned.

Leiper and Clapham (1938) recorded *T. retortaeformis* from two Chinese water deer, *Hydropotes inermis*, which were part of the stock of the Zoological Society of London at Whipsnade Park, Bedfordshire.

Sloan (1951) not only states that Leiper, in a private communication, mentions having found *T. retortaeformis* in a blackbuck, *Antilope cervicapra*, also from Whipsnade Park in 1937, but confirms that this nematode was recovered by him from one of three blackbuck which had died at Whipsnade Park during 1950-51.

T. retortaeformis is not the only member of the genus found in the grey squirrel in Britain since *T. colubriformis* has been reported from it by Baylis (1934 and 1939).

The occurrence of *Ascaris lumbricoides* in the squirrel is of interest but is not a new record. In the first edition of his textbook, Mönnig (1934) states that "the parasite has also been recorded from certain squirrels . . . " Rausch and Tiner (1948) not only found immature ascarids on three occasions in squirrels but quote Katz who reported *A. lumbricoides* from squirrels in 1938 as did Brown and Yeager in 1945. Rausch and Tiner did not consider their material adequate for definite specific determination but, in an addendum to their paper, they record the occurrence of *A. lumbricoides* from a fox squirrel from Wisconsin mentioning that the infection was probably of porcine, rather than of human origin. They further state : "We wish to call

the attention of those interested in the epidemiology of *Ascaris lumbricoides* that we still lack evidence that tree squirrels ever serve as reservoirs of infection. The small size of the lumen of the squirrel intestine as compared with the cross-sectional area of the adult worm would seem to preclude the chances of fertilized eggs being distributed on a very large scale. So far as we have been able to determine, there is no report of *A. lumbricoides* ova being found in any sciurid." The paucity of records of the occurrence of *A. lumbricoides* in squirrels shows that, in Britain, the grey squirrel is unlikely to act as a disseminator of this roundworm.

It now seems established that *Trichostrongylus retortaeformis* not only occurs in rabbits and hares in Britain but also in grey squirrels, at least in South East England, and that it has succeeded in adapting itself to ruminant hosts such as the goat, Chinese water deer and blackbuck. The possibility that the grey squirrel could act as a disseminator of this trichostrongyle to goats, as well as to rabbits and hares, cannot therefore be overlooked.

The writer wishes to express his thanks to many persons who sent or brought squirrel carcases for examination or who helped in other ways with this survey.

References

Baylis, H. A., 1934.—"XV. Miscellaneous notes on parasitic worms." *Ann. Mag. nat. Hist.*, **13** (74), 223–228. (W.L. 1050).

————, 1939.—"XXXVIII. Further records of parasitic worms from British vertebrates." *Ibid.*, **4** (23), 473–498.

Cameron, T. W. M. and Parnell, I. W., 1933.—"X. The internal parasites of land mammals in Scotland". *Proc. R. phys. Soc. Edinb.*, **22** (3), 133–154. (W.L. 16888).

Leiper, J. W. G., 1937.—"Natural Helminthiasis of the goat involving infection with *Trichostrongylus retortaeformis* of the rabbit." *Vet. Rec.*, **49** (45), 1411–1412. (W.L. 22523).

————, and Clapham, P. A., 1938.—"Some nematode parasites found in Chinese water deer (*Hydropotes inermis*), with a description of *Trichostrongylus cervarius* n.sp." *J. Helminth.*, **16** (2), 77–82. (W.L. 11224c).

Mönnig, H. O., 1934.—Veterinary Helminthology and Entomology. London. 1st Edition. p. 127.

Nagaty, H. F., 1932.—"The genus *Trichostrongylus* Looss, 1905." *Ann. trop. Med. Parasit.*, **26** (4), 457–518. (W.L. 1063).

Rausch, R. and Tiner, J. D., 1948.—"Studies on the parasitic helminths of the North Central States. I. Helminths of Sciuridae. *Amer. Midl. Nat.*, **39** (3), 728–747. (W.L. 640).

Sloan, J. E. N., 1951.—"A note on the occurrence of *Trichostrongylus retortaeformis* in the Blackbuck (*Antilope cervicapra*)." *Proc. zool. Soc Lond.*, **121**, 723–725. (W.L. 16969b).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 131–140.

On Two Rictularias and a Filariid from South African Wild Carnivores*

By R. J. Ortlepp
Veterinary Research Laboratory, Onderstepoort

Helminths from wild animals are from time to time submitted to Onderstepoort for identification and comment. Among this material are two lots of worms from carnivores which are of particular interest. One lot contains an undescribed *Rictularia* sp. from a Genet which is remarkable for the number of worms, over 30, collected from a single host ; as a rule the number of Rictularias collected from an individual animal is very limited consisting of no more than three or four specimens and then usually only females are present. The other lot from a mongoose is of interest because of the location of the worms—liver—and their number— about 60 ; this parasite also appears to be new to science.

In the ensuing pages a description of the two species mentioned above is given. It affords me the greatest pleasure to name one of the new *Rictularia* in honour of my esteemed and honoured teacher—Professor R. T. Leiper, F.R.S., who was instrumental in my taking up helminthology as a life's career; I shall always be greatly indebted to him for his encouragement, interest and friendship during my years of tenure as a research student and officer in his Department. It is through his teaching, guidance and inspiration that the beauty and marvels of these lowly animals have been revealed to me.

Rictularia leiperi sp. nov.

Material available :	11 males and 20 females all mature and collected from a single host.
Length :	Males 2·5 to 2·64 mm.; females 5·5 to 9·0 mm.
Breadth :	Males 0·192 to 0·228 mm.; females 0·372 to 0·43 mm.

Colour reddish brown in preserved material. Anterior extremity generally flexed ventralwards. Mouth directed dorsalwards ; body

* Published by permission of the Director of Veterinary Services, Onderstepoort.

finely annulated. Mouth capsule (Fig. 1 and 2) typical for genus with walls 0·009 mm. thick in females and 0·006 mm. thick in males ; dorsal free edge of capsule provided with about 15 small serrations acting as denticles ; ventral free border of capsule wavy, forming two hemispherical projections which in optical section, i.e. in lateral view of head, appear as two super-imposed pointed teeth from anterior (ventral) margin of mouth ; these correspond to the single large ventral tooth of *R. aethehini* le Roux. Dorsal and two subventral teeth at base of capsule, resting on anterior face of oesophagus ; dorsal tooth 0·01 mm. high and conspicuous in lateral view of head ; subventral teeth 0·008 mm. high ; dorsal gutter apparently not developed. Lateral diameter of capsule 0·06 mm. in females, 0·044 mm in males ; dorso-ventral diameter of capsule 0·072 in females, 0·042 mm. in males ; antero-posterior depth of capsule 0·036 mm. in females, 0·03 mm. in males ; all measurements include thickness of capsule. Four pairs cephalic papillae ; anterior (sub-ventral) pair on anterior lip ; lateral pair each a double papilla ; posterior (sub-dorsal) small ; in addition large dome-shaped papillae (amphid) lateral of sub-dorsal papillae.

Male. Body thickens posteriorly, maximum thickness just anterior to cloaca. Oesophagus in two parts, muscular and glandular ; glandular portion 0·55 to 0·62 mm. long by 0·084 to 0·102 mm. thick ; muscular portion 0·18 to 0·198 mm. long by 0·04 to 0·045 mm. thick. Nerve ring encircles muscular portion at its junction with glandular portion of oesophagus. Excretory pore not seen. Cervical papillae small and peg-like, some distance behind nerve ring, 0·36 to 0·396 mm. from anterior end, opposite 12th comb. Posterior extremity generally flexed ventralwards. Post cloacal region 0·16 to 0·18 mm. long, non-alate and ventral surface rugose. Sub-ventral combs consists of 64 or 65 pairs, seven of 11 males having 64 pairs and the remainder 65 pairs ; combs commence about 0·05 mm. from anterior end and extend through length of body, terminating just anterior to cloaca ; combs become larger posteriorwards and hindmost combs may reach length of 0·078 mm.; no true spines present, although posterior combs are more spine-like than those on anterior body half. Each male carries seven midventral fans, the posteriormost being largest and occupying oblique position across ventral surface ; remaining six fans arranged one behind the other along ventral mid-line ; anteriormost to posteriormost fans have respectively about the following measurements : 0·14 mm. long by 0·06 mm. high, 0·126 mm. by 0·046 mm., 0·09 mm. by 0·015 mm., 0·09 mm. by 0·015 mm., 0·106 mm. by 0·021 mm., 0·134 mm. by 0·033 mm. and 0·156 by 0·045 mm.

Eleven pairs sessile caudal papillae (Fig. 3), two pairs pre-cloacal, one pair ad-cloacal, four pairs post cloacal on anterior half of tail and four small pairs at posterior extremity of tail. Spicules simple, slender, pointed and equal, measuring 0·36 to 0·366 mm. in length by 0·006 mm. thick at their proximal ends. Gubernaculum absent.

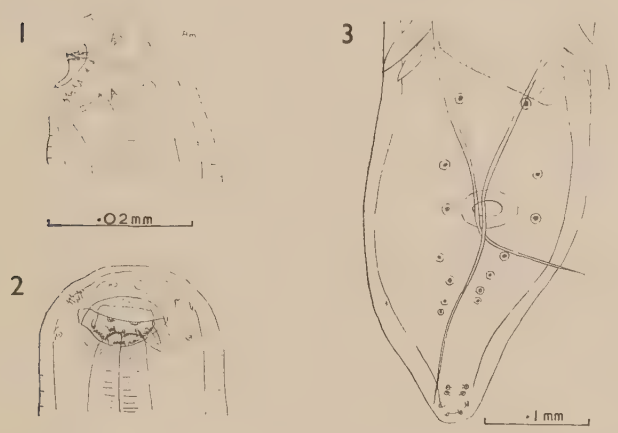


Fig. 1.—*Rictularia leiperi* sp. nov. Lateral view of head. **Am**=Amphid.
Fig. 2.—Dorsal view of head. Fig. 3.—Ventral view of male tail.

Female. Thickest portion of body in middle, tapers slightly posteriorwards, more attenuated anteriorly. Oesophagus in two parts, muscular and glandular, muscular portion 0·24 to 0·3 mm. long by 0·048 to 0·072 mm. thick, encircled by nerve ring at its base ; glandular portion 1·06 to 1·6 mm. long with maximum thickness of 0·102 to 0·156 mm. Cervical papillae opposite 11th or 12th comb, 0·54 to 0·63 mm. from anterior end. Excretory pore not located. Subventral combs and spines number 88 to 93 pairs of which the last 15 to 18 may be termed spines ; transition from combs to spines is very gradual ; these extend about 0·06 mm. from the anterior end to about 0·7 mm. anterior of anus ; largest combs about midway between anterior end and vulva and may reach a length of 0·156 mm. In 10 females, two had 28 combs, three had 29, and 5 had 30 combs anterior to vulva.

The vulva is non-protuberant and situated from 0·18 to 0·33 mm. posterior to junction of oesophagus and intestine. The two uteri practically fill up the whole of posterior two-thirds of body and contain numerous oval and embryonated eggs. Eggs smooth and thick shelled measuring from 0·04 to 0·042 mm. in length with a thickness of 0·027 to 0·03 mm. The tail is conical and is from 0·174 to 0·228 mm. long ; it is in all cases terminated by a small spike 0·007 to 0·012 mm. long.

Host : *Genetta felina* (Thunb.)

Location : Small intestine.

Locality : Hoopstad. O.F.S.

Types in Onderstepoort Helminthological Collection.

Affinities. The members of the genus *Rictularia* form such a homogeneous group that there are very few or no outstanding characters which might be considered to be of specific significance. This applies especially to the females, and where both sexes have not been available it is doubtful whether the creation of new species on females only has always been warranted. The task of sorting out the different species is in these cases rendered most difficult because usually the determiners have neglected to state on what characters they base their conclusions for the creation of their new species. Because of this difficulty the writer has compared the above described species only with those species where descriptions have been based on both sexes. The characters which the writer considers to be most important are collectively the total length of worms, the host, the presence (and number) or absence of midventral fans, the length of the spicules, the position of the vulva, the nature of the buccal armature, the number and extent of the cuticular combs in both sexes and the number and arrangement of the male caudal papillae. Of the described males, the only species from carnivores as far as the writer could ascertain, which have mid-ventral fans and spicules over 0·2 mm. in length are *R. paradoxuri* Tubangui and Masilungan, 1938, *R. affinis* Jaeg., 1904 and *R. splendida* Hall, 1913. The latter two species (which may be co-specific) each have eight mid-ventral fans, spicules about 0·2 mm. long, vulva posterior to the oesophagus and over 100 pairs of cuticular combs. The writer's species differs from them in having seven midventral combs, spicules up to 0·366 mm. long and the cuticular combs number less than 100 pairs. The first

named differs from the writer's species in possessing only four mid-ventral fans, and spicules about 0·1 mm. shorter ; it agrees with it, however, in the number of cuticular combs and in the position of the vulva. This species differs further from the writer's in that the males and females are about three times as large, and the male post-cloacal papillae number 10 pairs. There appears to be some relationship between the writer's species and *R. cahirensis* Jaegerskiold, 1904, but the writer has not been able to obtain any data regarding the species beyond the fact that the males possess seven midventral fans and the cuticular combs number up to 96 pairs in the male and 136 pairs in the female of which 46–52 are in front of vulva.

Specific diagnosis. Rictulariidae reaching a length of 2·6 mm. for the males and 9·0 mm. for the females. Mouth capsule with two ventral teeth and three oesophageal teeth ; dorsal gutter apparently absent. Males with 64 or 65 pairs cuticular combs, seven midventral fans, spicules up to 0·366 mm. long, and 11 pairs of caudal papillae. Females with 88–93 pairs of cuticular combs extending to about 0·7 mm. anterior to anus. Vulva post oesophageal, with 28 to 30 pairs of cuticular combs anterior to it. Eggs 0·04 to 0·042 mm. long by 0·027 to 0·03 mm. thick.

Rictularia myonacis sp. nov.

Material available. Ten females, from three mongooses, all fully mature. Length of mature females up to 15·2 mm.; breadth up to 0·372 mm. Head carries four pairs of papillae, of which all, except amphids, terminate in quarter-moon shaped somewhat chitinous structures ; lateral papillae single, not double as in *R. leiperi* ; buccal armature identical to that of *R. leiperi* except that oesophageal teeth are slightly larger. Combs and spines consist of 89–91 pairs extending to about 0·7 mm. anterior of the anus ; 43 to 44 combs anterior of vulva which lies 0·12 to 0·18 mm. posterior of oesophagus. Cervical papillae opposite 10th pair of combs. In two females (smallest and largest) muscular oesophagus 0·356 mm. long by 0·048 mm. thick and 0·408 mm. long by 0·06 mm. thick ; glandular oesophagus 2·12 mm. long by 0·096 mm. thick and 3·12 mm. long by 0·12 mm. thick ; nerve ring 0·27 and 0·33 mm. from anterior end ; tail 0·15 and 0·21 mm. long. Eggs 0·04 to 0·042 mm. long by 0·03 to 0·035 mm. thick.

The above described females differ from those of *R. leiperi* in being much longer, in having different type of cephalic papillae ending, in having lateral cephalic papillae with single ending and in having more cuticular combs anterior to the vulva.

It appears to have some affinities with the species *R. cahirensis* Jagersk., 1904 and *R. splendida* Hall, 1913, but differs from them in having fewer combs anterior to the vulva. A specific name is given to this species in order to avoid any future confusion.

Host : *Myonax cauui cauui* (A. Smith).

Location : Small intestine.

Locality : Onderstepoort, Transvaal.

Types in Onderstepoort Helminthological Collection

Hepaticofilaria pachycephalum gen. et sp. nov.

Material available. About three dozen males and same number females ; of the former about half were entire ; of females only one specimen was entire. *Length* : Males, 25 to 26 mm.; single entire female 55 mm. long. *Thickness* : Males, 0·144 to 0·156 mm.; females 0·3 to 0·324 mm.

Colour reddish. Body of more or less uniform thickness throughout, except for posterior extremity which becomes attenuated in both sexes. Cephalic extremity slightly thickened, cuticle with very indistinct annulations, only discernible on careful examination in some individuals. Mouth terminal, encircled by six cephalic papillae of which two lateral (? amphids), two sub-dorsal and two subventral (Fig. 4 and 6), enclosed by rectangular clear area, at corner of which an additional papilla appears to be present. Mouth leads into small inverted funnel-shaped buccal capsule with cuticular walls 0·004 mm. thick ; depth of capsule about 0·01 mm. and diameter at its base, including its wall, 0·016 mm. Characteristic depression in cuticle encircles anterior extremity, about 0·02 to 0·03 mm. from anterior tip. Oesophagus in two parts, muscular and glandular ; muscular oesophagus about same thickness throughout ; glandular oesophagus increases slightly in thickness posteriorwards. Nerve ring encircles muscular oesophagus just posterior to its middle. Excretory pore behind junction of two oesophageal parts. Cervical papillae very small, asymmetrical in position, about 0·05 mm. apart ; anteriormost papilla just anterior to level of excretory pore.

Male. Diameter of head 0·111 to 0·114 mm. Posterior extremity forms loose spiral. Muscular oesophagus 0·396 to 0·42 mm. long by

0·024 to 0·03 mm. thick; glandular oesophagus 1·13 to 1·12 mm. long with maximum thickness of 0·053 to 0·06 mm. Nerve ring 0·2 to 0·216 mm. from anterior end. Excretory pore 0·48 to 0·52 mm. from front. Anterior cervical papilla about 0·48 from front, posterior cervical papilla 0·53 to 0·56 mm. from front. Tail tapering 0·22 to 0·24 mm. long (Fig. 5). Caudal alae narrow 0·012 to 0·016 mm. broad. Four pairs of papillae and a single central unpaired papilla anterior to cloaca. Two pairs, in transverse row just behind cloaca, central pair smaller. A pair of ventral papillae at about junction of first and second tail thirds; three or four pairs of very minute

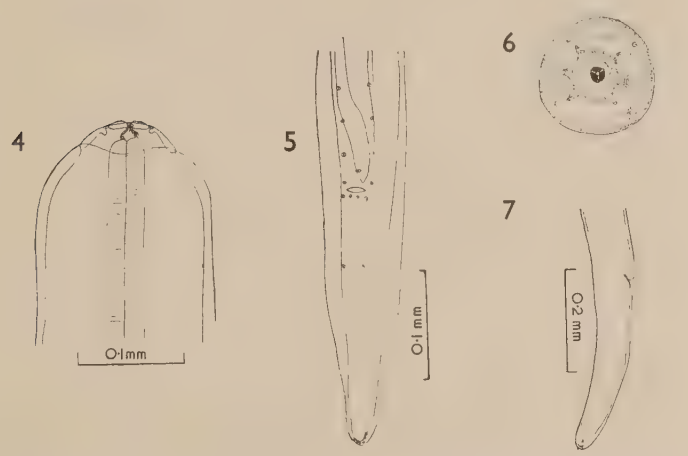


Fig. 4.—*Hepaticofilaria pachycephalum* gen. et sp. nov. Lateral view of anterior end. Fig. 5.—Ventral view of male tail. Fig. 6.—End-on view of anterior end. Fig. 7.—Lateral view of female tail.

papillae round tip of tail. Ventral surface of tail smooth, not rugose or ridged. Spicules dissimilar and unequal; left whiplike and slenderer than right which is shorter and has its posterior half enlarged and somewhat spatulate. Left spicule 0·375 to 0·4 mm. long by 0·012 mm. thick at its proximal end behind "flare"; its proximal portion, equivalent to handle of whip, 0·16 to 0·18 mm. long. Right spicule 0·144 to 0·168 mm. long by about 0·01 mm. thick at its proximal end and 0·018 to 0·021 mm. broad in its distal half. Gubernaculum absent.

Female. Thickness of head 0·156 to 0·163 mm. Muscular oesophagus 0·38 to 0·44 mm. long by 0·034 to 0·036 mm. thick. Glandular oesophagus 1·38 to 1·87 mm. long with maximum thickness of 0·054 to 0·06 mm. Nerve ring 0·24 to 0·26 mm. from anterior end. Excretory pore 0·57 to 0·59 mm. from anterior end. Anterior cervical papilla 0·57 to 0·58 and posterior cervical papilla 0·62 to 0·64 mm. from anterior end. Tail rounded, 0·3 to 0·312 mm. long and carries three small spines at its tip (Fig. 7) ; these spines 0·006 mm. long. Vulva in nearly all cases opposite or just behind middle of glandular oesophagus ; in a few cases the vulva was just posterior to oesophagus ; possibly this may be due to the fact that specimens were broken. Two uteri pass posteriorwards and practically fill whole body cavity. Viviparous and uteri filled with unensheathed embryos. Embryos 0·2 to 0·22 mm. long by 0·004 mm. thick. Anterior extremity bluntly rounded ; tail much attenuated. Both extremities appear smooth.

Host : *Ictonyx striatus* (Perry).

Location : Ducts (Veins) of Liver.

Locality : Houtbaai, Cape Town.

Types in Onderstepoort Helminthological Collection

Affinities : In that the females are not very much longer than the males, the absence of the peribuccal ring, the simple nature of the mouth without lips, the anterior position of the vulva and the presence of unequal and dissimilar spicules places this parasite in the sub-family Filariinae Stiles, 1907, of the family Filariidae (Cobbold, 1864). Among the genera of this sub-family its nearest approach appears to be to the genus *Litomosa* Yorke and Maplestone, 1926, with which genus it agrees in the presence of a small buccal capsule, whiplike nature of the longer spicule and absence of a gubernaculum. This genus differs from the writer's, however, in the absence of head papillae, non-annulated cuticle, and absence of caudal alae and papillae in the male. Consequently the genus *Hepaticofilaria* is created for its reception, which genus would be characterised as follows :—

Filariinae : *Females about twice as long as males. Mouth simple, no lips. Small buccal capsule present. Vulva in oesophageal region. Spicules unequal and dissimilar, longer whiplike. Caudal alae and papillae present in male. Gubernaculum absent.*

Type : *H. pachycephalum* from Mongoose, S. Africa.

Specific diagnosis : Reddish filaria, males up to 26 mm. long, females 55 mm. long or perhaps longer. Cuticular constriction near anterior extremity which is slightly enlarged. Left spicule 0.375 to 0·4 mm. long ; right spicule shorter 0·144 to 0·168 mm. long. Four pairs and one unpaired medium precloacal papillae ; three pairs postcloacal papillae. Three or four pairs of minute papillae at tip of tail. Tail coiled. Vulva generally opposite about middle of glandular oesophagus. Viviparous, larvae probably circulate in blood.

REFERENCES

BAYLIS, H. A., 1928.—"On a Collection of Nematodes from Nigerian Mammals, (chiefly Rodents)." *Parasitology*, **20,** 280–304. (W.L. 16035).

————, 1928.—"Some further parasitic Worms from Sarawak." *Ann. Mag. nat. Hist.*, ser. 10. **1,** 606–615. (W.L. 1050).

————, 1934.—"On a Collection of Cestodes and Nematodes from small Mammals in Tanganyika Territory." *Ibidem*, **13,** 338–353.

CHABAUD, A. G. and BRYGOO, E. R., 1956.—"Description de *Rictularia lemuri* n.sp. (Nematoda, Thelaziidae)." *Mém. Inst. sci. Madagascar, (ser. A—Biol. Anim.)*, **11,** 43–49. (W.L. 13251b).

———— and ROUSSELOT, R., 1956.—"Deux nouveaux *Rictularia* (Nematoda, Thelaziidae) d'Afrique Equatoriale." *Ann. Parasit. hum. comp.*, **31,** 225–265. (W.L. 899a).

CHANDLER, A. C., 1941.—"Helminths of Muskrats in South East Texas." *J. Parasit.*, **27,** 175–181. (W.L. 11428).

CUCKLER, A. C., 1939.—"*Rictularia onychomis* n.sp. (Nematoda : Thelaziidae) from the Grasshopper Mouse, *Onychomus leucogaster* (Weid.)." *Ibidem*, **25,** 431–435.

DOLLFUS, R. P. and DESPORTES, C., 1944/45.—"Sur le genre *Rictularia* Fröelich, 1802. (Nématodes Spiruroidea)." *Ann. Parasit. hum. comp.*, **20,** 6–34. (W.L. 899a).

DOUVRES, F. W., 1956.—"*Rictularia lucifugus* n.sp. (Nematoda, Thelaziidae from the little Brown Bat, *Myotis lucifugus lucifugus* (le Conte, 1831)." *Proc. helm. Soc. Wash.*, **23,** 40–47. (W.L. 16747a).

GALLI-VALERIO, B., 1932.—"Notes de Parasitologie et de Technique Parasitologique." *Zbl. Bakt.*, **125,** 129–142. (W.L. 23685).

GIBBS, H. C., 1957.—"The Taxonomic Status of *Rictularia affinis* Jägerskiold, 1909, *Rictularia cahirensis* Jägerskiold, 1909 and *Rictularia splendida* Hall, 1913." *Canad. J. Res. (Zool.)*, **35,** 405–410. (W.L. 5898c).

GOODRICH, A. L., 1932.—"*Rictularia scalopis* sp. nov. a Nematode from the Mole, *Scalops aquaticus* (Linn.)." *Trans. Amer. micr. Soc.*, **51,** 216–218. (W.L. 21400).

HALL, M. C., 1916.—"Nematode Parasites of Mammals of the orders Rodentia, Lagomorpha and Hyracoidea." *Proc. U.S. nat. Mus.*, **50,** 1–255. (W.L. 16944).

HOEPPLI, R., 1929.—"*Rictularia tani* n.sp. ein Parasit des Rattendarmes." *Zbl. Bakt.*, **110,** 75–78. (W.L. 23685).

JOHNSTON, T. H., and MAWSON, P., 1941.—"Some Nematode Parasites from Australian Birds." *Proc. Linn. Soc. N.S.W.*, **66,** 254–256. (W.L. 16790a).

KREIS, H. A., 1937.—"Beiträge zur Kenntnis parasitischer Nematoden IV. Neue und wenig bekannte parasitische Nematoden." *Zbl. Bakt.*, **138,** 487–500. (W.L. 23685).

LENT, H., and de FREITAS, J. F. T., 1935.—"Sobre dois novos Nematodeos parasitas da Quica—*Caluromys philander* (L.)." *Mem. Inst. Osw. Cruz*, **30**, 535–542. (W.L. 13465).

LE ROUX, P. L., 1930.—"A new Nematode (*Rictularia aethechini* sp. nov.), a Physaloptera and an Acanthocephala from the Hedgehog (*Aethechinus frontalis*)." *16th Rept. Div. vet. Serv. & Anim. Ind., Pretoria*, 217–232.

LOPEZ-NEYRA, C. R., 1956.—"Revision de la Superfamilia Filarioidea (Weinland, 1858)." *Rev. ibér. Parasit.*, **16**, 3–212 and 319–231. (W.L. 18858c).

————, 1957.—"Revision de la Superfamilia Filarioidea (Weinland, 1858). (Adiciones)." *Ibidem*, **17**, 169–273.

LUBIMOV, M. P., 1933.—"Rictularioseseuche bei Affen des Moskauer Tiergarten." *Zt. Infekkr. Haustiere*, **44**, 250–260. (W.L. 23460).

MACLEOD, J. A., 1933.—"A Parasitological Survey of the Genus *Citellus* in Manitoba." *Canad. J. Res.*, **9**, 108–127. (W.L. 5898c).

READ, C. P. and MILLEMAN, R. E., 1953.—"Helminth Parasites in Kangaroo Rats." *Univ. Calif. Publ. Zool.*, **59**, 61–80. (W.L. 22084).

SANDGROUND, J. H., 1933.—"Report on the Nematode Parasites collected by the Kelley-Roosevelts Expedition to Indo-China with Descriptions of several new species. I. Parasites of Birds. II. Parasites of Mammals." *Zt. Parasitenk.*, **5**, 542-583. (W.L. 23536b).

————, 1935.—"*Spirura michiganensis* n.sp. and *Rictularia halli* n.sp., two new parasitic Nematodes from *Eutamias striatus lysteri* (Richardson)." *Trans. Amer. micr. Soc.*, **54**, 155–166. (W.L. 21400).

SCHULZ, R. Ed., 1927.—"Zur Kenntnis der Helminthenfauna der Nagetiere der Union S.S.R. *Spirurata* Railliet & Henry." *Oeuw. de l'Inst. d'Etat. Med. Vet. Exper.*, Moscow, **4**, 36–39.

SEURAT, L. G., 1915.—"Sur les Rictulaires des Carnivores du Nord-African et les Affinités de genre *Rictularia*." *C. R. Soc. Biol. Paris*, **78**, 318-322. (W.L. 6630).

TINER, J. D., 1948.—"*Rictularia dipodomys* n.sp. (Nematoda : Thelaziidae) from the Kangaroo Rat, *Dipodomys* sp." *J. Parasit.*, **34**, 332–335. (W.L. 11428).

————, 1948.—"Observations on *Rictularia* (Nematoda : Thelaziidae) of South America." *Trans. Amer. micr. Soc.*, **67**, 192–200. (W.L. 21400).

TUBANGUI, M. A., 1931.—"Worm Parasites of the Brown Rat (*Mus norvegicus*) in Philippine Islands with special reference to those forms that may be transmitted to Human Beings." *Philipp. J. Sci.*, **46**, 537–591. (W.L. 16189).

———— and MASILUNGAN, V. A., 1937.—"Nematodes in the Collection of the Philippine Bureau of Science, III." *Ibidem*, **64**, 257–267.

YEH, L. S., 1959.—"A Revision of the Nematode Genus *Setaria* Viborg, 1795, its Host-parasite Relationship, Speciation and Evolution." *J. Helminth.*, **23**, 1–98. (W.L. 11224c).

YORKE, W. and MAPLESTONE, P. A., 1926.—The Nematode Parasites of Vertebrates. London.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 141–150.

Heterodera rostochiensis Population Density in Relation to Potato Growth

By B. G. Peters*
Imperial College, London, S.W.7

One fruitful way to explore the relationships between parasite and host is to expose the latter to a graded series of inocula of the former, and then to examine the effects of such treatment, both on the host and on the parasite.

An experiment of this type was reported by Lownsbery and Peters in 1955, in which tobacco plants were grown in pots of soil inoculated with *Heterodera tabacum* cysts at a geometric series of four densities from 0·5 to 32 cysts per gramme of soil. This happened to correspond to 50 to 3,200 larvae per g. Thus, the common factor was 4. Eight-fold replication, plus 8 controls (outside the geometric series) gave 40 pots in all. Half the pots at each density received sodium nitrate and limestone, so that an "inoculum × fertilizer" interaction could be studied.

Results showed that both heights (measured at 6 time intervals) and final weight were inversely proportional to the logarithm of the initial parasite density, each 10-fold increase in density leading to a 139 g. loss in plant weight and to a 51 cm. decrease in final height, for fertilized plants. Although data were given for final parasite densities, these were not analysed.

In the light of these linear relationships between height or weight of tobacco plants and log (density of parasite), Lownsbery and Peters were anxious to compare their *H. tabacum* findings with those for other species of *Heterodera*, and particularly for the closely related *H. rostochiensis*. They were unable to trace comparable published data. Accordingly, a somewhat similar pot experiment, but using *H. rostochiensis*, was carried out at Rothamsted in 1954.

Experimental Methods

Heterodera-free soil was mixed and 18·5 lb. (8·39 Kg.) weighed into each of 24 clay pots capable of holding 20 lb. when filled. An addition of 25 g. artificial fertilizers was made to each pot, measuring separately the N, P, and K salts of the mixture. From a previous

* Previously at Rothamsted Experimental Station.

experiment were available cysts of *Heterodera rostochiensis* of Vintage 1953 : there is some gain (in reduced variability) by using cysts of known age. The cysts were practically free from debris and were graded for size by using sieves of 30, 40 and 50 meshes per inch. The three size fractions were used in the same proportion to make up each of a geometric series of 5 inocula with a common factor of 4. Thus, the lowest inoculum was 213 cysts per pot, or about 0·025 c/g., or 1 cyst per 40 g. of soil ; and in the last terms the 5 inocula were : 1, 4, 16, 64 and 256 c/40 g. Each inoculum was in 4-fold replication, and 4 uninoculated pots were used as controls, making 24 pots in all. These last do not fall within the geometric series, but they have a possible use with plant criteria. There were 89·6 hatchable larvae per cyst in the inoculum, giving the c/g and 1/g values shown in Table 1.

TABLE 1

Means for inocula (x) and for treatment effects (y)

		Controls	Inoculum Density (c/40 g)				
		0	1	4	16	64	256
INOCULA							
(c/g)		—	0·025	0·101	0·405	1·62	6·48
(1/g)		—	2·26	9·05	36·2	145	579
EFFECTS							
H. (cm.)	38	16·25	18·5	17·0	14·5	13·75	11·0
	52	37·0	38·5	38·0	32·25	24·0	15·75
	66	43·0	43·75	43·75	40·0	32·0	17·0
	80	40·75	42·75	44·25	39·75	32·0	17·25
	94	42·0	44·25	44·5	40·75	32·0	15·75
Tuber No.		8·0	15·0	14·5	16·75	5·5	4·5
Tuber Wt.	(g)*	123	570	575	517	101	27·2
	(c/g)*	—	0·691	2·12	4·68	2·55	0·430
	(1/c)*	—	28·6	34·1	28·1	23·6	11·2
	(1/g)*	—	20·5	75·7	143	99·8	79·4

* Geometric means

Potato tubers of the variety Arran Banner were selected for even size (mean weight 71 g.), chitted, and all eyes but one obliterated. After planting a tuber in each pot (April 26th), the pots were randomized for position in an outdoor gravel plunge, where they stayed for 180 days.

Criteria

The criteria selected for measuring effects of treatment were of two kinds : plant-growth criteria and eelworm-population criteria. It has been shown before (Lownsbery and Peters, 1955) that the heights of tobacco plants at various times form a criterion which is sensitive to treatments of this kind. Potatoes have a different habit of growth but by interpreting height as "length of longest shoot" this criterion was applied fortnightly on days 38, 52, 66, 80 and 94 after planting. These criteria were analysed both as measured, and in logarithmic transformation (in order to reduce an observed correlation between mean and standard deviation). In the event the transformation made little improvement and the untransformed data are presented here.

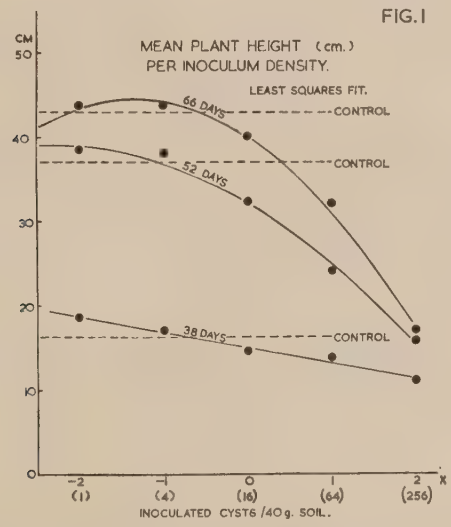


Fig. 1.—Relation between height of plant (cm.) and log inoculum, measured at 38, 52 and 66 days after planting.

Weight of crop is also a useful criterion, and here the logarithmic transformation was used. By also counting tubers it is possible to compute "weight per tuber"; this is sometimes of practical importance since a high weight of small tubers is of little economic value.

The eelworm criteria, based on cyst counts and counts of hatched larvae from samples taken at the end of the experiment, were all analysed in logarithmic transformation. In this case the treatment means are logarithms, and the corresponding antilogarithms are the geometric means of Table 1. Since counts of initial cysts and final cysts are both available, the difference will represent only the new cysts formed during the experiment. The criterion "hatched larvae per cyst" (l/c) is based on mixed new and old cysts, since the latter contribute some of the larvae. Accordingly the criterion "hatched larvae per g. of soil", which is the product of (c/g) and (l/c), must be based on "total cysts per g." instead of "new cysts per g."

Statistical Analysis

By plotting the observed criteria (y) against x=log (initial eelworm density), so as to convert the original geometric series : 1, 4, 16, 64, 256 into an arithmetic series (with equal intervals of log 4 in x), not only are the results usefully summarized in diagrammatic form but also it becomes possible to use the technique of orthogonal polynomials given by Fisher and Yates (1943). By this method, if the relationship between x and y is predominantly rectilinear, the "least squares" line is readily fitted whilst, if the relationship is curvilinear, a quadratic curve in x^2, or curves in higher powers of x, can be successively fitted. The test is whether the residual variance of observed points from the fitted line is, or is not, significantly larger than the basic error variance.

For example, the relationship between y=log (crop weight) and x=log (initial density) is shown in Fig. 2, based on data from Table 1. The corresponding analysis of variance of y is given below.

Term	Sum of Squares	D.F.	Mean Square
Linear	4·6199	1	
		} 2	2·7774
Quadratic	0·9350	1	
Cubic	0·0142	1	
		} 2	0·0639
Quartic	0·1137	1	
Error	1·6837	15	0·1123
Total	7·3665	19	

The computation is based on the use of Table XXIII of Fisher and Yates. Here the fitted straight line (Fig. 2) accounts for a reasonable proportion of the variability in tuber weight, but if a quadratic is fitted the fit is much closer and the residual variance of points from the curve is not greater than the error term.

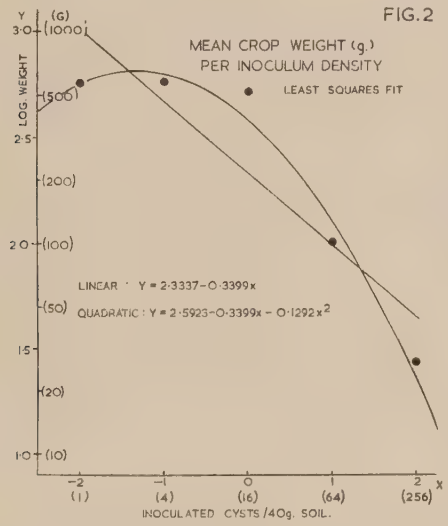


Fig. 2.—Relation between log (weight of crop) and log inoculum, with best-fitting linear and quadratic equations.

RESULTS

Treatment means are given in Table 1. Means for height in cm. at days 38 to 94, for No. of tubers harvested, and for cysts per g. and larvae per g. of soil in the inoculum, are all arithmetic means. The remainder are geometric means. The results are shown diagrammatically in Figs. 1 to 5, along with best-fitting (least squares) lines.

Plant Criteria

At day 38 (Fig. 1) the relationship between height and log (inoculum) was plainly rectilinear, as in the tobacco data of

Lownsbery and Peters (1955). The fitted line predicts that height (in cm.) will be given by

$$y = 14{\cdot}95 - 1{\cdot}825\,x. \qquad \text{(Unit of } x = \log 4\text{)}.$$

Thus there is a reduction of 1·825 cm. in height for each 4-fold increase in inoculum.

TABLE 2

Absolute and relative cyst-production

Inoculated Cysts/Pot x	New Cysts/Pot y	New cysts per cyst inoculated y/x
213	5,821	27·3
850	17,824	21·0
3,400	39,446	11·6
13,600	21,429	1·58
54,400	3,614	0·0663

TABLE 3

Realization of biotic potential : hatchable larvae (females)

Thousands/Plant		Percentage	
(a) ½ (inoculum) Potential♀♀	(b) New Cysts Actual♀♀	(c) 100.b/a Realization	(d) 100–c Wastage
9·5	5·8	61	39
38	18	48	52
152	39	26	74
6110	21	3·5	96·5
2438	3·6	0·15	99·85

At days 52 and 66 the data are best fitted by equations in x^2, thus :

$$\text{H.52} : y = 29{\cdot}70 - 5{\cdot}95x - 1{\cdot}29x^2.$$

$$\text{H.66} : y = 40{\cdot}19 - 6{\cdot}52x - 2{\cdot}45x^2.$$

At all three days the heights at low inoculations are slightly higher than in uninfested control plants. This was found by Lownsbery and Peters in tobacco at the lowest inoculum density of 50 l/g. In the present experiment there are three densities below 50 l/g., and at the lowest two of these the heights are more than in the controls.

After day 66 some of the shoots wilted and died, even among the controls, and there was no significant change from the situation at day 66, the growth of new shoots roughly compensating for the death of old.

The crop weights are shown in Fig. 2 where both a linear approximation and a closer quadratic fit are shown. One of the controls, the heights of which at the various times were comparable with those of the other three, produced no tubers at all. Omitting this anomaly the geometric mean weight was 123 g., considerably lower than the means at the lowest 3 inoculum rates. As in the previous tobacco data there is the suggestion of larger plants producing a larger crop at low inocula.

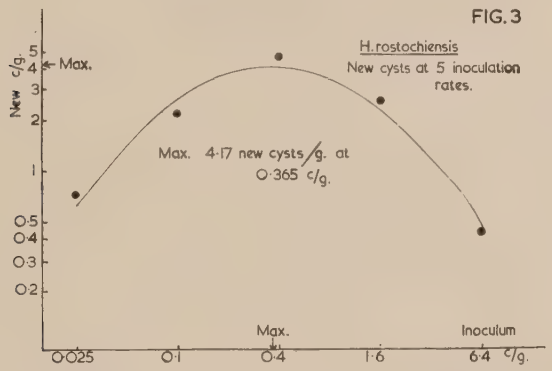


Fig. 3.—Relation between log (new cysts per g.) and log inoculum with best-fitting quadratic.

The fitted curve of Fig. 2 is of the type :

$$y = a + bx + cx^2.$$

Differentiating, $dy/dx = b + 2cx$.

On the curve y is maximal where $dy/dx = 0$ or $x = -1{\cdot}3154$. At this value of x the quadratic equation gives $y = \log(W) = 2{\cdot}816$. Under the conditions of this experiment a maximum yield of 654 g. should have resulted from an inoculum density of 0·0645 cysts per g. of soil, or one cyst per 15·5 g.

The "weight per tuber" criterion gave its highest value (43 g.) at the inoculum : 4 (c/40 g.), after which it fell steeply to only 7 g. at 256 (c/40 g.). The control value (omitting the tuberless plant)

was 28 g., so that here again the data favour a low inoculum for producing large tubers.

Eelworm Criteria

Table 1 and Figs. 3 to 5 illustrate the changes in the eelworm population at the various inoculum densities. Fig. 3 has been produced by plotting on a logarithmic scale the new cysts (taken as final cysts minus initial cysts). Logarithms of counts are used to reduce the correlation between mean and standard deviation but either direct counts or their logarithms show the same phenomenon of a small value at the lowest inoculum, rising to a maximum at about 0·4 (c/g.) and falling again to below the first value. The quadratic plot of Fig. 3 gives a maximum of 4·17 new cysts per g. of soil at an inoculum of 0·365 (c/g.).

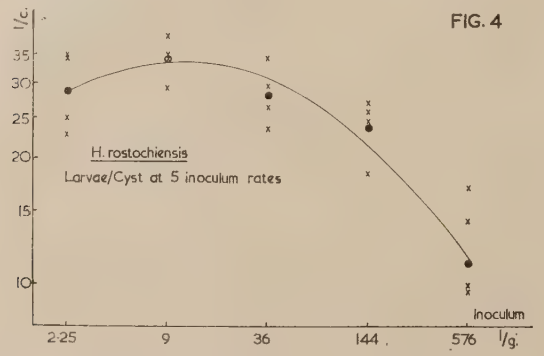


Fig. 4.—Relation between log (hatched larvae per cyst) and log inoculum with best-fitting quadratic. Inoculum : 89 (1/c).

Table 2 shows the initial and new cysts in terms of cysts per plant (or per pot), and their ratio : "new cysts per cyst inoculated". This brings out the expected result that multiplication is highest at the lowest inocula. It also shows that a single plant was at best able to support about 40,000 cysts.

Given the cyst situation, it is only to be expected that the final population of hatchable larvae per g. should also rise to a maximum and fall off. That the fall shown in Fig. 5 is not very marked is due to the effect of larvae still hatching from initial cysts also. Thus, at the highest inoculum a total of 6·8 (c/g) resulted of which only 0·4 (c/g) were new cysts.

The hatchable larvae per cyst of Fig. 4 (where individual values per pot are also shown to illustrate the degree of variability) display marked falling off at higher inocula. This is partly due to the reason already given, namely, that there are fewer new cysts and merely a residue of larvae from the old cysts.

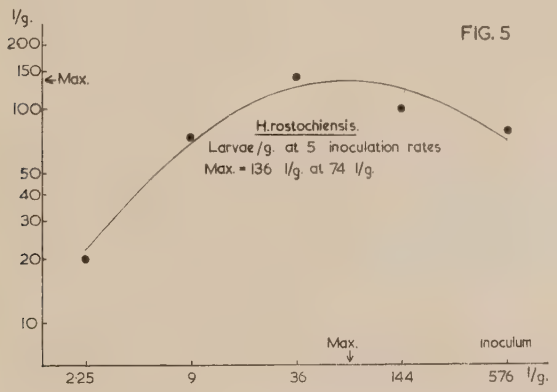


Fig. 5.—Relation between log (hatched larvae per g.) and log inoculum with best-fitting quadratic.

The basic measure of potential population is taken as hatchable larvae per g. of soil and here (Fig. 5) a maximum population of 136 (1/g) was predicted by the fitted curve, at an inoculum of 74 (1/g). The degree to which potentials of the initial counts are realized in the final counts are shown in Table 3, where the first column is based on the assumption that half the inoculated larvae are destined to become females. In the light of Ellenby's work (1954), this assumption is only approximately true. This Table suggests that the natural wastage at the lowest inoculum is surprisingly small (39%) but rises steeply to almost 100% at the highest inoculum.

Summary

By exposing potato plants in pots to a graded series of inocula of cysts of *Heterodera rostochiensis*, effects on the plant host have been investigated in terms of growth of shoots and tubers. High inocula reduce both criteria severely, but it appears that longer shoots and a

larger weight of larger tubers are produced by plants exposed to a very low inoculum than by uninfested controls.

Effects on final eelworm population show that multiplication is highest at the lowest inocula but that the net highest population results from a moderate inoculum. Thus, the kind of inoculum which would stimulate crop yields, would also lead to a large eelworm population : highest potato yields were associated with an eelworm multiplication factor of about ten-fold.

Acknowledgments

Most of the laboratory work and a good deal of the statistical analysis (including the orthogonal polynomial computations) were carried out under my guidance by Sibyl Cox whose skill, patience, and intelligence are gratefully acknowledged.

References

Ellenby, C., 1954.—"Environmental determination of the sex ratio of a plant parasitic nematode." *Nature, Lond.*, **174,** 1016. (W.L. 14900)

Fisher, R. A. and Yates, F., 1943.—Statistical Tables. London and Edinburgh, 2nd. Ed., pp. 20–22, 62.

Lownsbery, B. F. and Peters, B. G., 1955.—"The relation of the tobacco cyst nematode to tobacco growth." *Phytopathology*, **45,** (3), 163–167. (W.L. 16273)

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 151–156

The Physiology of Hatching of Eggs of *Ascaridia galli*

By W. P. Rogers
Department of Zoology, University of Adelaide, Adelaide, South Australia

According to Rogers (1958) the hatching of eggs of *Ascaris lumbricoides* involved several processes. A stimulus from the host caused the production of a "hatching fluid" within the infective egg ; the hatching fluid contained enzymes which attacked the egg-shell so that the embryo was released. This mechanism was also involved in the hatching of infective eggs of some other nematodes and in the exsheathment of infective third-stage larvae (Rogers, 1960). In both eggs and larvae the stimulus from the host was dependent upon the concentration of undissociated carbonic acid and dissolved gaseous carbon dioxide, the oxidation-reduction potential and the hydrogen-ion concentration. A more detailed account of these and some other factors which stimulate the hatching of eggs of *Ascaridia galli* are given here.

Methods

Adult female worms were incubated in saline for 24 hours at 37°C. The eggs were collected and their stickiness reduced by washing them for 1 hour in 0·5N sodium hydroxide or by incubating them in 1% pancreatin at *p*H 6·8 for 6 hours. Thereafter they were incubated at 28°C for 16 days in 0·5% formalin or 0·1N sulphuric acid. They were stored in water at 5°C until required for experiments. Some eggs were dried at 28°C at 100% humidity before they were used.

Bicarbonate-carbon dioxide buffers were prepared according to Umbreit, Burris & Stauffer (1957) and checked with a glass electrode. All solutions were brought to the appropriate *p*H before they were added to buffers. Care was taken to exclude oxygen from solutions when sodium dithionite was used. Unless stated otherwise experiments were carried out at 37°C for 4 hours. The concentration of undissociated carbonic acid plus dissolved gaseous carbon dioxide was calculated using the formula given by Rogers (1960).

Except where stated otherwise experiments were repeated at least three times with different lots of eggs. Though the results obtained with different lots of eggs varied somewhat the general trends were consistent except in experiments in which the effect of the concentration of carbon dioxide in the gas phase on hatching was examined. These experiments were therefore repeated with 10 lots of eggs.

Results

The Effect of Hydrogen-ion Concentration.—The hatching of eggs was examined in bicarbonate-carbon dioxide buffers containing 0·02M sodium dithionite and with a gas phase of 1% carbon dioxide in nitrogen over the range *p*H 5 to *p*H 8. At the low end of the range the buffering action was weak but the *p*H could be maintained between 4·8 and 5·3. The other buffers were maintained within $\pm$*p*H 0·1. One set of results is shown in Fig. 1 (solid line). It should be noted that factors besides *p*H could have affected the hatching of the eggs. Thus the osmotic pressure of the buffers rose with the *p*H; the concentration of sodium bicarbonate at *p*H 5 was about 1×10^{-4}M and it rose to $114 \times ^{-4}$M at *p*H 8. The concentration of undissociated carbonic acid and dissolved gaseous carbon dioxide also varied with the *p*H. At *p*H 5 the total concentration of these components was about 2×10^{-4}M. At *p*H 8 it was $0{\cdot}05 \times 10^{-4}$M.

The Effect of Sodium Chloride.—The hatching of eggs in buffers at *p*H 8 (gas phase 1% carbon dioxide in nitrogen) containing the reducing agent was measured at different osmotic pressures. The addition of sodium chloride at concentrations up to 0·2M did not greatly affect the hatching of eggs (Fig. 1, broken line). At 0·4M hatching was inhibited. In one experiment only the effect of adding sodium chloride at different concentrations to compensate for the different osmotic pressures due to sodium bicarbonate over the range *p*H 6 to 8 was examined. The sodium chloride had little effect under these conditions.

It seems unlikely then that the increased hatching of eggs with increases in *p*H was due to increases in the total electrolyte concentration.

The Effect of Carbon Dioxide.—The hatching of eggs was measured at *p*H 8 in $M/_{100}$ phosphate buffer under nitrogen and in bicarbonate-carbon dioxide buffers with gas phases of 0·1%, 1%, 5%, 10% and 20% carbon dioxide in nitrogen. Sodium dithionite, 0·02M, was present.

Ten different lots of eggs were examined in this way. Though the results with each lot of eggs were generally consistent, the results varied with different lots of eggs. Concentration of undissociated carbonic acid plus dissolved gaseous carbon dioxide above $0{\cdot}05 \times 10^{-3}$M always inhibited hatching at *p*H 8. In six experiments most eggs hatched when the concentration was between $0{\cdot}5 \times 10^{-6}$M and $0{\cdot}5 \times 10^{-5}$M. On two occasions most eggs hatched when nitrogen without carbon dioxide was used for the gas phase. Eggs which had been dried for several days after they had become infective often hatched better at higher concentration of undissociated carbonic acid plus dissolved gaseous carbon dioxide than did the eggs which had been kept wet, but this difference was not consistent.

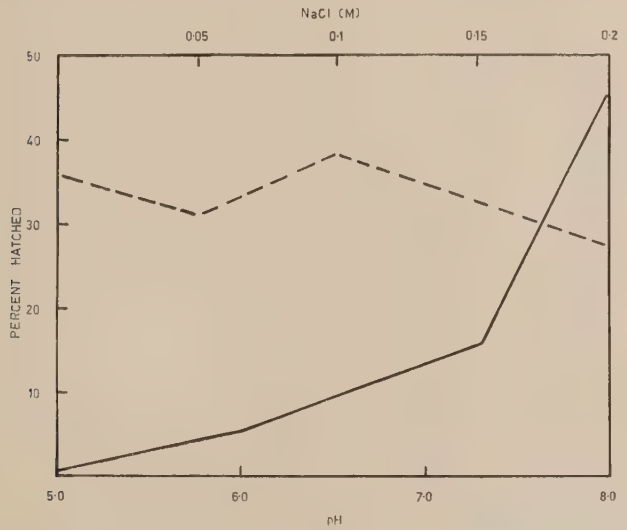


Fig. 1.—The relationship between *p*H and the hatching of eggs in bicarbonate-carbon dioxide buffers containing 0·02M sodium dithionite with a gas phase of 1% carbon dioxide in nitrogen (solid line).

The effect of adding sodium chloride on the hatching of eggs (broken line). The gas phase was 1% carbon dioxide in nitrogen and the *p*H was 8·0.

The effect of carbon dioxide on the hatching of eggs at higher hydrogen-ion concentrations has not been examined in detail but results show, as with the eggs of *Ascaris lumbricoides* (Rogers, 1960), that the concentrations of undissociated carbonic acid plus dissolved gaseous carbon dioxide which inhibited hatching increased as the *p*H was decreased.

The Rate of Hatching of Eggs.—In bicarbonate-carbon dioxide buffer containing 0·04M sodium dithionite under 5% carbon dioxide in nitrogen few eggs hatched during the first hour of incubation. Thereafter the proportion of eggs which hatched rose rapidly. The results of one experiment are shown in Fig. 2 (broken line); in two other experiments the relative proportions of eggs which hatched after two hours was smaller.

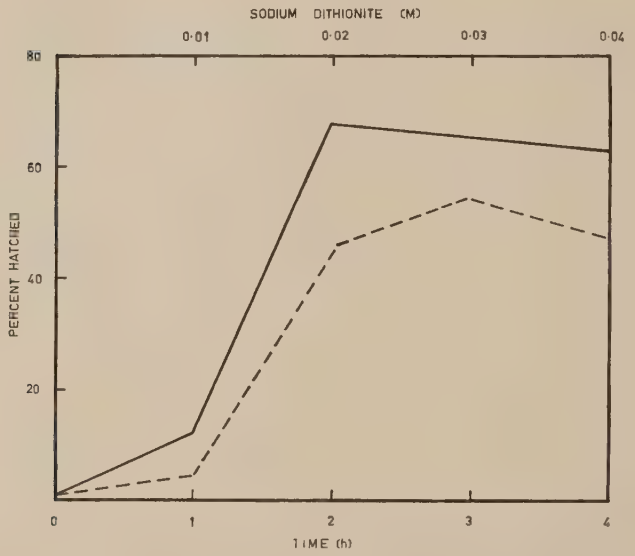


Fig. 2.—The relationship between the concentration of sodium dithionite and the hatching of eggs in bicarbonate-carbon dioxide buffer under 5% carbon dioxide in nitrogen at *p*H 8·0 (solid line).

The time of hatching of eggs in bicarbonate-carbon dioxide buffer containing 0·04M sodium dithionite under 5% carbon dioxide in nitrogen at *p*H 8·0 (broken line).

The Effect of Reducing Agents.—In bicarbonate-carbon dioxide buffers with carbon dioxide in nitrogen as the gas phase the addition of sodium dithionite increased the hatching of eggs. The efficacy of the reducing agent varied with different lots of eggs. Sometimes it increased hatching greatly (Fig. 2 solid line). At other times the proportion of eggs which hatched without the reducing agent was high (up to 32%) and this was doubled in the presence of 0·02M sodium

dithionite. The optimum concentration of sodium dithionite was 0·02 to 0·04M (Fig. 2 solid line).

The effect of other reducing agents, ascorbic acid and cysteine, was examined. At 0·02M cysteine was often more effective than sodium dithionite. Ascorbic acid was less effective than the other two reducing agents.

Discussion

The stimulus for the hatching of eggs of *Ascaridia galli* was similar to that needed for *Ascaris lumbricoides* (Rogers, 1958, 1960). It seemed that lower concentrations of undissociated carbonic acid plus dissolved gaseous carbon dioxide were more effective for *Ascaridia galli* and inhibition of hatching occurred at lower concentrations at *p*H 8. But evidently these differences are unimportant. Thus the infective eggs of *Ascaris lumbricoides* were found to hatch in the small intestine of both mice and chickens (Rogers, unpublished). The eggs of *Ascaridia galli* hatched well in the chick ; they also hatched in the mouse but whether this host is as suitable as the chicken for the hatching of eggs is as yet uncertain. Certainly the abnormal host was less suitable for the emerged larvae. In the chick, for instance, sometimes as many as 75% of the larvae of *Ascaris lumbricoides* which hatched within 4 hours after dosing were dead.

The optimum conditions for the hatching of eggs of *Ascaridia galli* varied somewhat with different lots of eggs especially in respect to the concentration of undissociated carbonic acid plus dissolved gaseous carbon dioxide. At first it was thought that this might have been due to the method of preparing the eggs. However other methods did not give more uniform lots of eggs. The *Ascaridia galli* used for this work were obtained from birds which had been grown and killed for commercial purposes and it was not possible to standardise the conditions under which the worms were collected. This may have caused some variations in the eggs.

The hatching of eggs of *Ascaris lumbricoides* seemed to be the result of the indirect action of the stimulus (Rogers, 1958). The actual breakdown of the egg-shell was due to the production of chitinase and other enzymes which attacked the membranes of the egg. It is not known if the same process occurs in the eggs of *Ascaridia galli* but as the stimulus is similar it probably does.

Summary

The stimulus for the hatching of infective eggs of *Ascaridia galli in vitro* depended upon the concentration of undissociated carbonic acid plus dissolved gaseous carbon dioxide, the oxidation-reduction potential, and the hydrogen ion concentration. There was considerable overlap in the conditions which favoured the hatching of eggs of *Ascaridia galli* and *Ascaris lumbricoides*; both would be expected to hatch in the small intestine of a suitable species of host.

Acknowledgments

I wish to express my indebtedness to Professor R. T. Leiper, F.R.S. who first encouraged me to undertake research work on the physiology of parasites. For many years Professor Leiper has taken an interest in my work and has given me helpful advice.

I wish to thank Mrs. M. Ross for skilled assistance. Most of the apparatus used in the work was provided by the Rockefeller Foundation.

References

Rogers, W. P., 1958.—"Physiology of the hatching of eggs of *Ascaris lumbricoides*." *Nature, Lond.*, **181**, 1410. (W.L. 14900).

———, 1960.—"The physiology of infective processes of nematode parasites; the stimulus from the animal host." *Proc. roy. Soc.*, **B152**, 367. (W.L. 16900).

Umbreit, W. W., Burris, R. H. and Stauffer, J. F. 1957.—Manometric Techniques. Burgess Publishing Co., Minneapolis.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 157–160.

On a New Species of the Genus *Chevreuxia* Seurat, 1918 from India

By S. N. Singh, D.Sc. (London)
Professor of Zoology, Osmania University

and

S. M. Ali, Ph.D. (Osmania)
Reader in Zoology, Osmania University

Four male specimens of this species were once collected from the white necked Stork, *Dissoura episcopus episcopus*. The worms occurred under the horny layer of the gizzard.

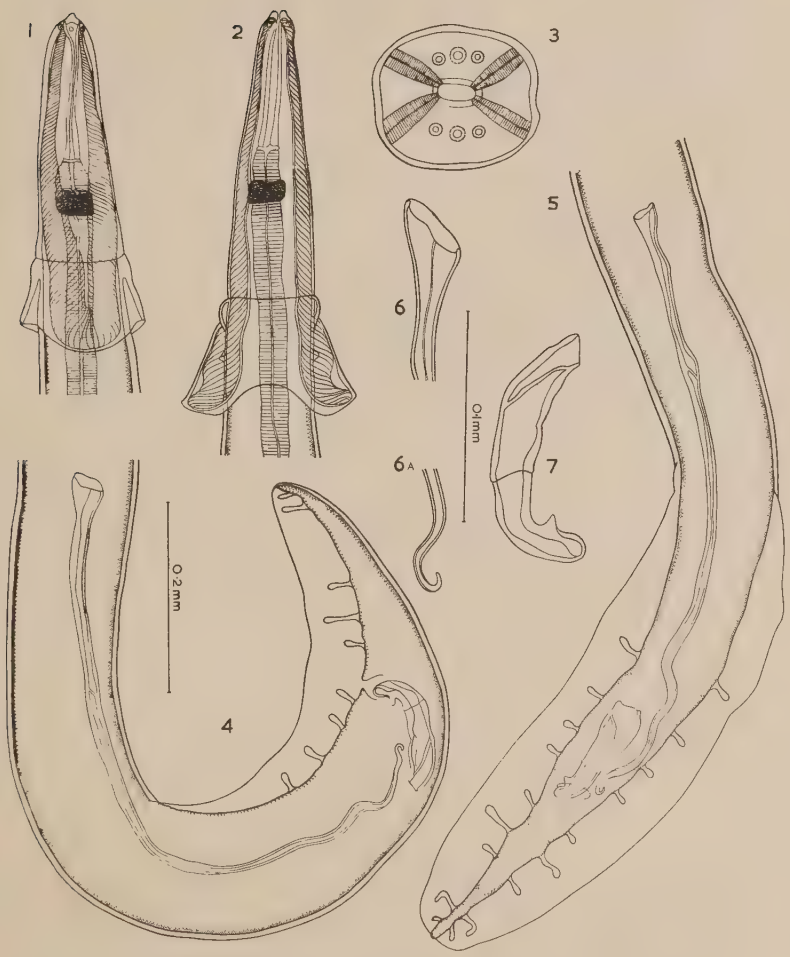
These are rather thin worms with a cylindrical body having tapering ends. The specimens measure 15·8–18·9 mm. in length and 0·16–0·18 mm. in width. The cuticle is thick with fairly distinct transverse striations. Surrounding the cervical papillae, which are situated at a distance of 0·31–0·35 mm. from the anterior tip of the head, is a trapezium shaped hyaline sheath of cuticle, encircling the body—a characteristic feature of the genus. The hyaline collarette starts at a distance of 0·24–0·28 mm. and extends up to 0·34–0·41 mm., from the head end. The collarette is 0·090–0·094 mm., and 0·13–0·18 mm. wide at its anterior and posterior borders respectively. There are two pairs of dorsal and ventral cordons, joining with their counterparts laterally, just in front of the posterior border of the collarette, at a distance of 0·34–0·41 mm. from the anterior end. The cordons are well developed arising in pairs between the bases of the lips, and increasing in width as they run posteriorly. The blunt conical lips are fairly prominent, each bearing a pair of cephalic papillae and a laterally situated amphid. The nerve ring and the excretory pore are located at 0·19–0·20 mm. and 0·21–0·23 mm. from the anterior end. The mouth opens into a funnel shaped vestibule which is 0·16 mm. long. The oesophagus measures 3·22–3·77 mm. in length, occupying $^1/_5$ of the entire body length. Its two component portions measure 0·79–0·85 mm. and 2·43–2·92 mm. respectively.

The conical tail which is 0·21–0·24 mm. long, is surrounded by well developed alae, 0·61 mm. long and 0·159 mm. wide. There are altogether 9 pairs of pedunculated caudal papillae, of which 4 pairs are preanal and the rest postanal. The postanals are arranged in two groups of 3 and 2 pairs each. The second pair of the papillae of the former group is much larger than the other two. In the second postanal group situated near the tip of the tail, the penultimate pair of papillae is much larger. The precloacals are evenly spaced and of almost the same length.

The spicules are very unequal in length and dissimilar in shape. The left spicule is 680–910μ long with a broad anterior end, and an inwardly curved tip. The right spicule is strongly chitinised and is 139–181μ long. It is navicular in shape with a broad obtusely curved posterior end, and with a prominent spine-like projection just anterior to its tip. There is a transverse ridge-like thickening at a little distance behind the middle of its body. The ratio between the two spicules is 5 : 1.

Discussion. The genus *Chevreuxia* was erected by Seurat in 1918 for Rudolphi's *Spiroptera revoluta*, 1918. Since then only one new species *Chevreuxia australis* was added, by Johnston and Mawson in 1941. The present worm is the third species, being reported from India. Of *Chevreuxia revoluta*, both the males and the females are known. Johnston and Mawson established their new species on a single female specimen. The description of the species reported herein, is based on four male specimens only.

The present worm seems to be the longest of the three species, the length of the males exceeding even that of the females of the other two. It differs from *C. australis* also in the position of the cervical papillae and the shape of the collarette. The collarette starts from behind the cervical papillae in *C. australis*, whereas the cervical papillae are situated in the middle of the collarette in the present worm. The males of *C. australis* being unknown and the characters of the female on which the new species is established being "slight" (Johnston and Mawson, 1941), the present worm could only be compared with the genotype. The males of *C. revoluta* are almost one third as long as those of the worm under discussion, in which moreover there is the characteristic spine-like projection on the right spicule which is absent in *C. revoluta*. In addition to these, various other body measurements differ in the two species. It is therefore concluded that the worm described above, forms a new species of the



Chereuxia leiperi sp.nov. (male only)

Fig. 1.—Anterior extremity, lateral view. Fig. 2.—Anterior extremity, dorso-ventral view. Fig. 3.—*En face* view of head. Fig. 4.—Lateral view of tail. Fig. 5.—Ventral view of tail. Fig. 6.—Proximal end of left spicule. Fig. 6a.—Distal end of left spicule. Fig. 7.—Right spicule.

genus *Chevreuxia*. It is proposed to name it *C. leiperi* in honour of Professor R. T. Leiper.

Host : *Dissoura episcopus episcopus*

Habitat : Gizzard.

Locality : Hyderabad, Andhra Pradesh, India.

The type specimens are deposited in the Zoology Museum of Osmania University.

References

Cram, E. B., 1927.—"Bird Parasites of the Nematode Suborders Strongylata, Ascaridata and Spirurata". *Bull. U.S. nat. Mus.*, **140**. (W.L. 5659).

Johnston, T. H. and Mawson, P. M., 1941.—"Additional Nematodes from Australian birds". *Trans. roy. Soc. S. Aust.*, **65**, 254–262. (W.L. 21654).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 161–168.

Investigations on the Anti-bacterial Properties of Infective Larvae of Some Parasitic Nematodes

By W. Stefański and Z. Przyjalkowski

Institute of Parasitology, Polish Academy of Sciences, Warsaw

Contrary to the opinion expressed in various manuals of parasitology and pathology, cases of germ-carrying by the larvae of parasitic worms from the intestines of an organism, remain extremely rare. Similarly the intestinal mucosa of the intestines damaged by parasites does not necessarily form "a gate through which takes place infection" of bacteria and viruses. This is additionally proved by experimental research conducted in the first place by Taylor (1935), Enigk (1954), Stefański (1956), as well as by Stefański and Zebrowski (1958) and also by Przyjalkowski (1958).

The simplest explanation of this phenomenon was based on the conjecture that the secreta and excreta of parasitic worms have bacteriostatic or bactericidal properties. The authors were entitled to put forth such a conjecture as a result of the work of several authors, such as James and Mandoul (1906), Joyeux (1907) and especially that of Jettmar (1952 and 1953).

The demonstration of bacteriostatic or bactericidal properties in larval parasites should partly explain the fact of non-transmission of bacteria and viruses by infective larvae, hence the present investigation was undertaken.

Material

Infective larvae of the following species or nematodes groups were used in the experiments : *Ascaris suum* ; *Oesophagostomum dentatum*; *Strongyloides ransomi* ; *S. papillosus* ; infective larvae of intestinal and stomach nematodes of sheep together with larvae of *Dictyocaulus filaria* ; infective larvae of Strongylinae and Trichonematinae from horses and infective larvae of *Trichostrongylus retortaeformis* from hares. With the exception of the infective larvae of nematodes from intestines and stomach of sheep and horses, which were submitted to group tests, all the larvae were examined separately for each species of parasite.

Preparation of Test Material

Infective larvae of *Ascaris suum* were obtained from the lungs of mice and guineapigs which were previously fed on a culture of this parasite's eggs. The larvae from the lungs were obtained by Baermann's method. Larvae of other nematodes were bred in laboratory conditions from eggs obtained from animal faeces known to contain a sufficient quantity of eggs.

Faeces required for the culturing of eggs were taken directly *per rectum*. Faeces of sheep and pigs were mixed in equal parts with faeces of horses, sterilized for two hours in an autoclave under 2 atm. pressure.

The lightly pressed culture of faeces was placed in a crystallizer at a temperature of about 20°C. for 6–10 days. It was given a daily airing and was provided with the required degree of humidity. When the infective larvae appeared they were immediately isolated from the faeces by Baermann's method, a pipette was used to collect the Strongylinae and Trichonematinae larvae in the droplets of moisture settling inside the cover of the crystallizer containing the faeces culture. These water drops contained thousands of larvae unsoiled by any particles of faeces. The larvae were then washed in a normal saline solution with detergent and spun in an electric centrifuge at about 1000 rev./min. Sterilized liquid soap was used as detergent in doses of two drops of soap per 15 ml. of the normal saline solution containing the larvae.

Methods

All experiments intended to ascertain the anti-bacterial properties of infective larvae for a given animal species, were conducted mainly on bacteria pathogenic for this species, but also on those non-pathogenic bacteria which were already established as being susceptible to anti-bacterial action of extracts from mature parasites. The experiments were carried out on twenty species of bacteria.

The experiments were conducted according to the diffusion plate method, namely by using a cylinder plate method, a streak assay, or by cutting a hollow in the agar in the middle of the plate, as well as by the serial dilution method, in sampling tubes with simple nutrient broth.

Isolated bacteria strains were obtained from the Collection of Strains of the Polish Academy of Sciences in Warsaw and Pulawy. Twenty-four hour old cultures from simple nutrient broth were used in the experiments.

Cylinder plate method

Well clarified 1·5–2 per cent ordinary agar of *p*H 7·0 was used in the cylinder plate method. The agar was poured on Petri dishes, measuring 100 mm. × 20 mm. and five glass cylinders were placed in each dish. They measured 12 mm. long, 7 mm. inside diameter and were of 1 mm. thick glass. They were all polished on the end as they were to be sunk into the agar layer. All cracked cylinders, or those with rough or uneven surfaces, were rejected.

Cylinders standing upright on the Petri dish were sterilized in an electric hot-air oven, and while still hot, were at once placed on plates with agar previously sown with bacteria cultures and cooled for a period of 24 hours. They were placed on the rim of the plate at equal distances from the middle of the plate. The hot cylinders melted the agar and sank evenly into it by their own weight ; the molten agar compound hardened instantly round the cylinder forming with it a kind of cup or small basin. This method permits one to test the antibiotic even in non-sterilized fluids. For each species of bacteria, three plates with five cylinders each were used. To three of the cylinders the tested substance was introduced by drops, the fourth one remaining free ; and in the fifth, fluid obtained from the body cavity of mature specimens of *Ascaris suum* was placed. All cylinders were then filled up to half of their height. The 4th and 5th cylinders served as control of the agar nutrient.

Streak assay method

In the streak method, which presents the advantage of testing 5–6 bacteria strains on the plate, the agar and the dimensions of the plates used were the same as in the cylinder plate method. A furrow, 80 mm. long, 30 mm. deep and 10 mm. wide was cut in sterile agar. The furrow was cut below the centre of the plate so that the ends of it reached almost to the rim of the plate. Using a platinum loop, six different strains of bacteria were sown, in the form of straight dashes, running parallel to each other but vertically to the furrow and on the larger area of the agar. The furrow was then filled up with the sterile extract of the larvae to be tested.

Agar hole method

In this method a central hole is cut out in the agar in the middle of the plate. The hole was 10 mm. in diameter and 3 mm. deep. Only sterile fluids can be tested according to the last two methods in view of the overgrowth of bacteria contained in non-sterile fluids.

Serial dilution method

In the serial dilution method ordinary nutrient broth was used. The extract made from larvae was used in dilutions of 1:10, 1:20, 1:40, 1:80 and 1:100. Washed in normal saline solution nematode larvae were ground in a Weigel mortar, then a small quantity of normal saline solution was added to give the mixture a fluid consistency. The mixture was then placed in a refrigerator at a temperature of 1°C. for twenty-four hours.

Procedure

The material prepared from larvae was then used for tests according to the "cylinder plate" method and sometimes also according to the "agar hole" method*. For control purposes live larvae were also introduced into the cut-out hole together with the extract obtained from ground larvae. The tracks of the larvae migrations were often visible on the total surface of the agar plate, thus giving evidence of larval vitality.

For the remaining methods and particularly for the serial dilution method, extracts of larvae were prepared. Larvae washed as above were dried at a temperature of 37°C. in a thermostat. After thorough drying the larvae were ground to powder on a mortar and the powder was diluted with normal saline solution. The obtained suspension was then stored at a temperature of 1°C. for eight days and was submitted to repeated shakings many times every day. After that the suspension was centrifuged and filtered through a Seitz filter. The obtained extract was checked for its sterility.

* "Agar hole" method—a shortened name which will be used on later pages for definition of the "central hole cut out of the agar in the middle of the plate" method.

Results of Experiments with Larvae of Various Nematodes

Ascaris suum

Investigations conducted on larvae of this parasite excited the authors' keenest interest because of the bactericidal properties, stated by Pavan and Jettmar, of the perivisceral fluid, taken from the cavity of the body of the mature *Ascaris suum* as well as of some others of their organs, in regard to the Gram-positive bacteria.

Investigations were carried out on *Erysipelothrix rhusiopathiae, Eberthella typhosa, Salmonella typhimurium, Sarcina lutea, Micrococcus lysodeicticus, Staphylococcus albus, Staphylococcus aureus, Staphylococcus citreus, Streptococcus equi* and *Brucella abortus bovis* (Bang). All experiments conducted according to the cylinder plate, streak assay or "agar hole" methods *gave no evidence of inhibiting growth of the mentioned bacteria.*

Strongyloides ransomi and *Strongyloides papillosus*

In view of the difficulty of obtaining infective larvae of *Strongyloides ransomi* in substantial quantities, the experiments were carried out only according to the cylinder plate method in relation to the following bacterial strains—*Erysipelothrix rhusiopathiae, Salmonella typhimurium, Eberthella typhosa, Pasteurella suiseptica, Sarcina lutea* and *Micrococcus lysodeicticus.*

In order to confirm the obtained results, further experiments were conducted on the sheep parasite *Strongyloides papillosus.* The experiments were carried out according to the cylinder plate method in relation to the following bacterial strains—*Erysipelothrix rhusiopathiae, Salmonella typhimurium, Eberthella typhosa, Escherichia coli, Sarcina lutea* and *Staphylococcus albus* and gave no evidence of any anti-bacterial properties of the sheep parasite *Strongyloides papillosus.*

Oesophagostomum dentatum

Research on the larvae of this nematode obtained from eggs bred in faeces of infected pigs was carried out according to the cylinder plate, streak assay and "agar hole" methods. The following bacteria strains were used—*Erysipelothrix rhusiopathiae, Sarcina lutea, Streptococcus equi, Salmonella typhimurium, Escherichia coli, Pseudomonas pyocyanea.* The growth of none of these strains was inhibited.

Intestinal and Stomach Nematodes of Sheep

Investigations carried out with these nematodes in relation to *Erysipelothrix rhusiopathiae, Sarcina lutea, Escherichia coli* and *Salmonella typhimurium* yielded negative results.

Strongylinae *and* Trichonematinae *of Horses*

The larvae of the above mentioned nematodes obtained simultaneously from faeces of horses infected with horse strongyles were used in the experiment. As large quantities of these larvae were obtained, the experiments were carried out according to all the methods mentioned previously. The experiments covered the highest number of bacterial strains, namely, eighteen. Every strain was submitted to a thrice repeated test by each of the methods applied. Tests according to the serial dilution method were carried out twice. The following bacterial strains were used—*Erysipelothrix rhusiopathiae, Staphylococcus albus, Staphylococcus aureus, Staphylococcus citreus, Micrococcus lysodeicticus, Sarcina lutea, Streptococcus equi, B. subtilis, B. mesentericus, B. megeatherium, Escherichia coli, Eberthella typhosa, Salmonella typhimurium, B. pseudodiphteria, Serratia marrescens, Pseudomonas pyocyanea, Actinobacillus equuli* and *Brucella abortus bovis* (Bang). None of the used bacteria strains proved to be sensitive to the extract from ground-up larvae, to living larvae, to substances secreted by the larvae or to extracts from the respective groups of larvae.

Trichostrongylus retortaeformis

Investigations carried out in 1935 by Taylor aiming to solve the problem whether larvae of migrating nematodes are carriers of bacteria from the intestines of an organism were conducted with larvae of the nematodes *Trichostrongylus retortaeformis* and *Graphidium strigosum*. Taylor proved that the larvae of the aforesaid nematodes do not bring about *Salmonella choleraesius* infection from the lumen of the intestine of rabbits. As the authors succeeded in obtaining *Trichostrongylus retortaeformis* larvae by breeding a culture of eggs obtained from faeces of infected hares and from mature females of the above mentioned parasite, they checked Taylor's experiment on the point as to whether his results were not due to antibacterial elements developed by the larvae used for the experiment. The experiments were carried out with *Salmonella choleraesius, Salmonella typhimurium* and *Eberthella typhosa*. It was eventually proved that also in this case the growth of the above mentioned bacteria was not inhibited either by the extract of ground-up larvae or by live specimens of *Trichostrongylus retortaeformis* larvae.

CONTROL TEST RESULTS

A characteristic zone of inhibition of growth appeared around cylinders No. 5, filled with perivisceral fluid taken from the cavity of mature *Ascaris suum*, in relation to the Gram-positive bacteria strains used. On the other hand, around cylinders No. 4 (empty) no traces of such a zone were observed. These control tests prove that diffusion took place in the agar used for the experiments and that the components of agar have no hampering influence on the growth of bacteria.

CONCLUSIONS

The fact that the growth of bacteria was not inhibited in the tests proves that there are no antibacterial factors in the larvae used.

If it is accepted that the larvae have no antibiotic influence on bacteria with which they come in contact when passing from the intestines to the tissues of the host, then it must also be accepted, on the basis of the results of experiments indicating that bacteria are not transmitted by infective nematode larvae, that the infection by bacteria in these cases is inhibited by some immunity factor of the host organism and especially by the immunity reactions of the tissues. The relatively frequent instances of infection by germs brought about by nematode larvae through the skin, which apparently was a weaker barrier against the bacteria than the intestinal mucosa, may serve as indirect proof of this theory.

REFERENCES

ABRAHAM, E. P., CHAIN, E., FLETCHER, C. M., GARDNER, A. D., HEATLEY, N. G., JENNINGS, M. and FLOREY, H., 1941.—"Further observations on Penicillin." *Lancet*, **241**, 177–189. (W.L. 11996).

FLEMING, A., 1946.—"Penicillin—Its Practical Application." 75–92.

FOSTER, J. W. and WOODRUFF, H. B., 1943.—"Microbiological aspects of Penicillin." *J. Bact.*, **46**, 187–202. (W.L. 11056).

GAUZE, G. F., 1956.—"Lekcii po antibiotikam". Moskwa.

HITCHCOCK, D., 1948.—"The inhibition of Bacteria by the metabolic products of Trichomonas." *J. Parasit.*, **34**, 113–117. (W.L. 11428).

JAMMES, L. and MANDOUL, H., 1906.—"Tenias et flore intestinale". *C. R. Soc. Biol., Paris*, **58**, 229–230. (W.L. 6630).

JETTMAR, H. M., 1952.—"Ueber die bakteriostatische und baktericide Wirkung der Coelomfluessigkeit der Ascariden." *Z. Hyg. InfektKr.*, **134**, 24–46. (W.L. 23454).

———, 1953.—"Ueber die bakteriostatische Wirkung der Ascariden-Cuticula." *Arch. Hyg. Berl.*, **136**, 568–573. (W.L. 1770).

JOYEUX, CH. and BAER, J. G., 1929.—"Étude sur le reencapsulement der *Sparganum ranarum*." *C. R. Soc. Biol. Paris*, **101**, 305–307. (W.L. 6630).

PAVAN, M., 1952.—"Antibiotica tierischer Herkunft. Antibakterielle Wirkung parasiter Eingeweidewuermer." *Z. Hyg. InfektKr.*, **134**, 136–161. (W.L. 23454).

SCHMIDT, W. and MOYER, A. J., 1944.—"Penicillin. Methods of Assay". *J. Bact.*, **47**, 199–208. (W.L. 11056).

STEFANSKI, W., 1955.—"Stosunki biocenotyczne pomiedzy fauna pasozytnicza i flora bakteryjna." *Kosmos, seria "A", Biolog.*, **1**, 13–21.

————, 1956.—"Les relations biocénotiques entre la faune parasitaire et la flore bacterienne du tract digéstif. II. Le rôle des helminthes dans la transmission du rouget du porc." *Acta Parasit. Polon.*, **4**, 521–554.

————, and ZEBROWSKI, L., 1958.—"Investigations on the transmission of Newcastle disease virus by *Ascaridia galli* and the pathogenic synergism of both agents." *Bull. Acad. Polon Sci., série des Sci. Biol.*, **6**, 67–72.

PRZYJALKOWSKI, Z., 1958.—"Investigations on the transmission of *Salmonella typhimurium* in Animals by migrating nematode larvae." *Ibidem*, **6**, 383–388.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 169–174.

Favored RLE for Axenic Culture of *Neoaplectana glaseri*

By Norman R. Stoll
The Rockefeller Institute, New York

The value of RLE (raw liver extract) as a supplement in promoting the development of the parasitic nematode, *Neoaplectana glaseri* Steiner, 1929, in axenic cultures of infusion broth has been earlier noted (Stoll 1951, 1953a), as has the enhancement of results through utilizing extracts made from livers of animals late in pregnancy or early postpartum (1954a, b, 1959). Herewith are summarized experiments over a 2½ year period in which this enhancement was apparent with RLE derived from rabbit livers.

Material and Methods

Preparation of heat labile RLE has been described, with the axenic culture methods used for *N. glaseri* (Stoll 1953a, 1959). Standardization for the present comparative tests included the use of RLE made from livers of the source and handling indicated. It was employed as a 1 ml. supplement with 9 ml. heat stable infusion broth, in tube cultures inoculated with 25 3rd-stage larvae, and the tubes examined for total yield of worms after being kept in a shaker for 21 days at 22°C., as noted below.

Livers. Rabbits from the Rockefeller Institute stock colony, a New Zealand-Chinchilla cross inbred for 13 years, were sacrificed, half in the period February to May, and half July to September. After excision from the exsanguinated donors, the livers, with gall-bladders removed, were refrigerated promptly and then frozen. The tissue was held at –20°C. from ½ week to 51 weeks (median 6) before use, protected in the freezer from excess loss of moisture by careful wrapping in wax paper and aluminium foil.

RLE. Liver tissue, thawed in the refrigerator overnight, was triturated in an electric blender in the ratio 100 grams to 400 ml. triple-distilled water at 7–11°C. The brei, after titration in the cold to *p*H 4 with normal HCl, was centrifuged, sometimes after initial paper filtration, to eliminate the non-fluid portion. It was the rule to keep all processing below 15°C., but occasionally in this series that temperature was transiently exceeded by 5–9° at centrifugation (an unwelcome factor later avoided by using a refrigerated centrifuge). The clear fluid after sterilization by Seitz filtration, with a *p*H of about 4·5, was then transferred in 20 ml. amounts to 22×180 mm.

tubes for refrigeration. In the present cultures it was used after 1 to 688 (median 60) days storage.

Inoculum. The strain of *N. glaseri* in use was isolated in 1944 from grubs of the Japanese beetle, *Popillia japonica* Newm., and maintained in stock cultures of fresh sterile kidney tissue on dextrose agar slants (Glaser 1940, Stoll 1953a, 1954b). It was demonstrated to have retained its parasitic character and pathogenicity for its natural host after 7 years such serial transfer *in vitro* (Stoll 1953b), and while not tested further in beetle grubs, had (and has since) shown no change in cultural characteristics.

Larvae to inoculate cultures were fished with a heavy bacteriological loop from the walls of the stock culture tubes, to which they migrate as 3rd-stage forms. Until used in cultures they were stored in the refrigerator, in lots of several thousands per 50 ml. Erlenmeyer flask, in sterile distilled water of 1 cm. depth.

For inoculation an aliquot of larvae from a storage flask was first transferred by Pasteur pipette to a 15 ml. pointed graduated tube and dilution counted. Additional transfers to similar graduated tubes with sterile water were made until sufficient larvae were available in subtubes to inoculate all cultures in a given experiment, each inoculum to be 25 active larvae in 0·5 ml. withdrawn from the suspensions. The final subtubes received sterile dextrose solution in amount suitable to provide 25 mg. dextrose per inoculum.

A high degree of consistency was obtained in the number of larvae inoculated by carefully resuspending them in the water before removing a 0.5 ml. sample that had been quickly delimited to its size. This is illustrated by a test in which 4 such larval suspensions, respectively of 5, 6, 8 and 10 ml., earlier adjusted to produce 58 similar inoculations, were completely sampled with regular sterile technique in 0.5 ml. amounts, and all 58 withdrawals counted. A mean of $24{\cdot}1 \pm 0{\cdot}2$ resulted, with 37 of the 58 samples showing 23, 24 or 25 larvae ; extremes were 14 and 27 ; the C.V. was 8·3 per cent.

The inoculations used for 46 of the 47 experiments containing cultures in the present RLE tests, were of larvae stored not over three months ; the one exception utilized larvae 7 months old.

Cultures. Infusion broth (Rockefeller Institute formula) made from fresh beef hearts, with 1 per cent peptone (Pfanstiehl) added, was titrated to *p*H 6·0, and Arnoldized. After transfer in 9 ml. amount to culture tubes 22×180 mm., this was supplemented with 1 ml. RLE, which produced little *p*H change. The tubes were inoculated with the 0·5 ml. larval suspension containing dextrose and placed in racks in a shaker that had a 2 cm. traverse and operated

at 100 strokes a minute. (Decreased shaking through lower rate or shorter traverse produced decreased yields; when not shaken at all the resulting average yields were decreased one-third). The shaker room, kept dark, was temperature-controlled at 22°C. Cultures were shaken for 21 days, during which 3 generations of *N. glaseri* developed.

Counting the Yield. Cultures, begun with 10·5 ml. fluid, had usually evaporated to about 9 ml. in 3 weeks in the shaker. They were made up with sterile water to a previously placed 9 ml. or 10 ml. mark, and dilution sampled by removing 0·5 ml. of the thoroughly stirred contents using a S-C pipette for transfer to a graduated tube. (The S-C is a Pasteur pipette made from 8 mm. OD Pyrex glass tubing of standard wall thickness, drawn so that with a final overall length of 27 to 29 cm., a long taper permitted a graduation of 0·5 ml. to be marked at 5 to 7 cm. from a tip of 2·0 mm. ID. A constriction placed about 3 cm. from the 8 mm. end, made for convenience in cotton plugging. Both ends were flame smoothed without constriction of lumen. After sterilization, the S-C pipette was capped with a rubber bulb positioned so that release of full pressure on the bulb allowed fluid to quickly fill the pipette to the 0·5 ml. mark.)

The 0·5 ml. from the culture being examined was made up to 5 ml. with water, and the diluted suspension sampled by withdrawing 0·5 ml. for transfer to a $1\frac{1}{2} \times 3$ in. glass slide on which a large area with double-line border had been outlined by means of a "China marker" pencil to restrain the uncovered drop. All the worms were counted under low power of the microscope (5× ocular with 22·7 mm. objective giving ×30 magnification) with the aid of a mechanical stage, and a tally counter. If the yield was low, 0·5 ml. directly from the culture was countable without dilution. Cultures with large numbers of worms required sub-dilution so that the most favorable counting range of 50 to 100 worms on the slide was approximated. Two or more samples of 0·5 ml. were examined from each dilution tube; for large populations, duplicate second dilution series were made from the culture.

Sterility. As is required for true axenic cultures, no antibiotic, bacteriastatic, bactericidal or fungicidal agents were employed (Stoll, 1959). All components before use, and the media after culture, were routinely tested for sterility in beef-heart-infusion-peptone broth, and occasionally also in thioglycollate broth and on Sabouraud dextrose-agar slants. When contamination occurred in our axenic routine about half were moulds, usually single per culture. Cultures lost through both moulds and bacteria represented about 6 per cent of those set into 42 of the experiments involved in this study,

approximately normal expectation under our conditions of operation ; in 5 additional experiments there occurred the axenicist's nightmare of a loss of over half the tubes.

Results

The 595 cultures summarized in Table I for comparison of RLE were parts of 47 experiments, about half of which were performed in 4 months of a 2½ year period. In 22 of these experiments, 2 to 9 (median 4) RLE were compared *inter se* ; in the 25 others one RLE only was represented. The individual experiments furnished data on 2 to 10 cultures (median 4) per RLE.

Reference to the last two columns of the Table shows two series of mean yields of worms that developed from inocula of 25 larvae in 3 weeks. Mean yield I is derived from the individual RLE averages presented in the column just preceding, and were from cultures initiated when the RLE had been refrigerated 15–90 days (see below). Mean yield II is similarly derived from all other cultures made from these RLE (not all were fully represented as indicated in the table footnotes), initiated with RLE less than 15 or more than 90 days old.

These I and II means both indicate the same general result, that there was an increase to more than double the yield of worms from cultures in which RLE was derived from rabbit livers the donors of which were late in pregnancy or early *postpartum*. The effect was soon lost, those donors 17–60 days *postpartum*, even though the does were still lactating, furnishing RLE of about the value of non-pregnant rabbits. No enhancement effect was demonstrated in RLE made from livers of newborn young. Adult male donors tested were in the general range of unbred females.

A positive correlation of yield in relation to period of refrigerated storage was noted in the course of the above experiments. RLE, when transferred to tubes after passing through the Seitz filter, is characteristically serum-like in appearance. After refrigeration at 2–4°C. for one to two weeks, a precipitate forms which may resemble a clot-like cast slumped in the bottom of the tube. This precipitate is resuspended when RLE is introduced into cultures. If supplemented with RLE before the precipitate formed, cultures were lower in yield than after it had formed. A preliminary analysis of the data indicated a period of optimum potency 15 to 90 days after the RLE was made, with lessened value thereafter.

An expression of this change can be given, despite the different numbers of cultures available for testing the different RLE, and of their inherently different yield "efficiency". If the mean yield of

all cultures made during the 15–90 day period with one RLE is treated as the optimum yield (100 per cent), that of each and every culture obtained from the use of that RLE can be stated as a percentage of this optimum. The percentages can then be grouped as frequency distributions in terms of how long the RLE had been kept

TABLE I

Yields of *Neoaplectana glaseri* at 3 weeks from axenic cultures inoculated with 25 3rd-stage larvae each, in relation to RLE supplement classified as to rabbit donor source for sex, age, and pregnancy. Series I from RLE refrigerated 15–90 days ; Series II from RLE stored less than 15 or more than 90 days.

RLE groups	Donor rabbits	Number tested	Av. worm yield : thousands (Cultures furnishing av.)					Mean yield I of RLE groups	Mean yield II (Extra cultures)
A	Non-pregnant	4	3·5	4·1	4·9	5·2		4430	3570[b]
			(3)	(27)	(8)	(19)			(45)
B	Pregnant	3	5·1	6·1	8·8			6670	6400[c]
	1 week		(15)	(9)	(15)				(10)
C	Pregnant	5	3·6	3·9	4·1	10·3	11·9	6750	6670[b]
	2 weeks		(5)	(19)	(5)	(4)	(4)		(37)
D	Pregnant	4	2·1	3·0	4·9	9·0		4750	5150
	3 weeks		(7)	(13)	(4)	(12)			(36)
E	Pregnant	3	9·5	10·1	12·7			10,800	8300[c]
	4 weeks		(16)	(4)	(12)				(43)
F	Postpartum	4	7·8	8·1	14·8	16·9		11,900	10,300[c]
	1–7 days		(5)	(5)	(8)	(11)			(34)
G	Postpartum	4	2·5	2·8	3·8	4·7		3450	3870
	17–60 days		(4)	(11)	(20)	(12)			(25)
H	Newborn	2[a]	1·5	1·9				1700	1750
	young		(8)	(7)					(4)
I	Adult male	4	3·8	5·5	5·6	6·8		5430	3970
			(10)	(10)	(8)	(10)			(31)
	Total	33 RLE	330 cultures						265 cultures

[a] 2 litters, of 4 and 5 young, unclassified for sex ; livers from littermates were pooled.
[b] 3 RLE groups only.
[c] 2 RLE groups only.

before use. When this was done (using, for decreased percentage variability, cultures from RLE that had mean yields of 5,000 worms or more in the optimum period), the following percentage relationships, with probable errors, resulted for yield of worms from RLE stored before use as specified :

RLE stored 1–7 days, 69·3±2·8 per cent.
,, ,, 8–14 ,, , 89·5±2·8 ,, ,,
,, ,, 15–30 ,, , 98·4±3·8 ,, ,,
,, ,, 31–60 ,, , 100·5±2·2 ,, ,,
,, ,, 61–90 ,, , 98·8±2·2 ,, ,,
,, ,, 91–120,, , 71·8±2·5 ,, ,,
,, ,, 121 and over 59·5±2·3 ,, ,,

No positive correlations were found in analysing the data of yield in relation to :

1. Period of year in which the animals were sacrificed ;
2. Size of donor animal, or size of livers (excluding newborn young) ; or
3. Period of frozen storage of liver before processing.

Discussion

The results indicating increased value for axenic *N. glaseri* cultures of RLE made from livers of rabbits in late pregnancy or early *postpartum*, as judged by increased populations of worms developing in such cultures under comparable conditions, have been abundantly verified in experiments other than those shown, (and also by Dr. G. J. Jackson working in this laboratory), but not included because of modifications in the standardization noted in obtaining the present data.

Summary

Favored RLE for use in promoting growth and development of *Neoaplectana glaseri* in axenic cultures include those made from the livers of rabbits sacrificed late in pregnancy or early *postpartum*, and used after subsequent refrigerated storage periods of 15–90 days.

Acknowledgment

Appreciation is expressed for the technical assistance of Patricia White de Lorenzo and Carol Claflin Wurtz.

References

Glaser, R. W., 1940.—"The bacteria-free culture of a nematode parasite." *Proc. Soc. exp. Biol.*, **43**, 512–514. (W.L. 16913).

Stoll, N. R., 1951.—"Axenic *Neoaplectana glaseri* in fluid cultures." *J. Parasit.*, **37**, 18. (W.L. 11428).

———, 1953a.—"Axenic cultivation of the parasitic nematode, *Neoaplectana glaseri*, in a fluid medium containing raw liver extract." *Ibidem*, **39**, 422–444.

———, 1953b.—"Continued infectivity for Japanese beetle grubs of *Neoaplectana glaseri* (Nematoda) after seven years axenic culture." *Thapar Commemoration Volume*, Lucknow, 259–268.

———, 1954a.—"Improved yields in axenic fluid cultures of *Neoaplectana glaseri* (Nematoda)." *J. Parasit.*, **40**, 14. (W.L. 11428).

———, 1954b.—"Further progress in the axenic cultivation in fluid media of *Neoaplectana glaseri*, a parasitic nematode." *Anat. Rec.*, **120**, 745–746. (W.L. 763).

———, 1959.—"Conditions favoring the axenic culture of *Neoaplectana glaseri*, a nematode parasite of certain insect grubs." *Ann. N.Y. Acad. Sci.*, **77**, 126–136. (W.L. 1051).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 175–178.

Acquired Immunity and Helminthic Disease

By E. L. Taylor

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge

Resistance against an infective organism is a self evident requirement for healthy survival within the endemic area of any spreading disease, the most constant and the most effective force being the immunity that is acquired as a result of infection. In developed parts of the world most members of the human community must go through the process of acquiring resistance to measles, mumps and chicken pox in order to have assurance that they will not at some time be incapacitated by one or the other. The dog community is in a similar position with regard to distemper. This principle for preparation to live healthy in an infected environment is widely accepted for the microbial crowd—diseases of man and animals, but has not so far received general acceptance for diseases caused by parasitic worms.

The strongyloid diseases of grazing animals form a particular example of this tardy acceptance. For a number of years the phenomenon of immunity to helminths in general has been regarded as of little more than academic interest, a faint reflection, in fact, of the dramatic demonstrations that could be given by the bacteriologists. Although latterly the power of this response in maintaining

the health of communities in the field has been recognised by those few parasitologists who have interested themselves in the epidemiological aspect of these diseases it has needed the production of a vaccine (2) to bring the point to wider notice.

The epidemiological relationships between worm parasites and disease outbreaks among communities of hosts are now seen to be the same as those of bacterial or viral diseases and their host communities. The factors in operation—susceptibility, infection, dissemination and protection—are therefore identical and it seems reasonable, therefore, to apply to the field of helminthology such principles of epidemiology as have been accepted by the bacteriologists and virologists, and so derive benefit from their conclusions. These guiding principles are regrettably few, but they appear to be adequate to show that several of the very generally accepted ideas about the control of strongyloid diseases have been wrong in their aim.

Detailed investigations carried out some thirty years ago, (1 and 3) both in Great Britain and in the United States, using large communities of mice, and infections of suitable bacteria and viruses, led to the recognition of three main types of epidemic :— (1) those occurring when a virulent strain of organism gains access to a previously unexposed population, (2) those occurring in infected communities when dosage is increased by some environmental factor, and (3) those occurring in infected communities when population resistance is lowered. It was also concluded that the parasite will never completely disappear from a large infected group of animals.

If this classification of epidemic types be applied to the occurrence of strongyloid disease among sheep and cattle as they are managed on farms we may clearly see type (1) where calves are carefully protected from infection in the farm buildings, and then, as wholly susceptible animals are placed on infected pasture, at an age when they are able to eat a considerable amount of grass and so take in a large dose of infective larvae. The same type may be seen where milking cows that have been using a succession of clean ley pastures for months, and sometimes for years, are placed onto pastures that have been used by some of the infected younger animals. It may also be seen in the practice of strip grazing, or of the folding of lambs, whereby the first time over the crop the movement of the feeding animals is always onto clean ground. In this way they avoid the benefit of the gradual acquisition of increasing infestation, and

when they proceed over the same crop a second time they suddenly come across a heavy infection that they cannot withstand.

The second type of epidemic, (2) is, however, the one most commonly seen in helminthic disease among grazing animals. Susceptible calves, for example, or lambs are crowded onto pasture which they proceed to infect, and, because they are crowded together they increase the rate of infection more quickly than they are able to develop their resistance. Type (3) is less prevalent, although examples can be found where store cattle are kept alive during the autumn and winter on a sub-maintenance diet.

The resistance which is acquired by young animals and reinforced by repeated contact in adult life makes adequate provision for the maintenance of health in an infected environment and the above examples clearly show its paramount importance where parasites are so widely distributed as are the strongyloid nematodes of our grazing animals. Up to the present time, however, it has not received adequate recognition. The avowed aim in control has continued to be the protection of young animals from all contact with the parasite, and the production of infection-free pastures, in order that particularly susceptible, or particularly valuable groups of animals may be segregated there.

It may be argued that, while the farmer endeavours to keep his animals free from infection he can never succeed, and that he only reduces to a reasonable and useful level the infection that is sure to be there. The rationale of the situation ought, however, to be clearly recognised by the expert, whose reasoned advice should be the exposure of young animals to infection at an early age, and the spreading of the parasites over the whole farm. This would bring about the timely acquisition of immunity by young animals that are entering into the infected flock or herd, and avoid the production of susceptible groups of animals kept in temporary isolation within infected areas. It is agreed that the most advantageous rate of infection for the development of immunity in young animals is a difficult point to determine, but it seems reasonable to recommend that the production of infection—free areas should be avoided in farming practice.

References

Greenwood, M., Hill, A. B., Topley, W. W. C. and Wilson, J., **1936.**—"Experimental Epidemiology". *M.R.C. Rep.* **209.**

Jarrett, W. F. H., Jennings, F. W., McIntyre, W. I. M., Mulligan, W. and Sharp, N. C. C., 1959.—"Studies on immunity to *Haemonchus contortus* infection—vaccination of sheep using a single dose of X-irradiated larvae." *Amer. J. vet. Res.*, **20,** 257–531. (W.L. 628a).

Webster, L. T., 1930.—"The Rôle of Microbic Virulence, Dosage and Host Resistance in Determining the Spread of Bacterial Infections among Mice." *J. exp. Med.*, **52,** 901–948. (W.L. 11189).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 179–186.

The Life History of *Olveria indica*, an Amphistome Parasite from the Rumen of Indian Cattle

By Gobind Singh Thapar

In 1945, Thapar and Sinha reported the occurrence of an interesting new genus and species of Amphistome, *Olveria indica*, from the rumen of cattle and buffaloes in Uttar Pradesh. Later Tandon (1951) recorded a second species of this genus from buffaloes from Lucknow slaughter houses. Thapar (1956), in reporting on the incidence and intensity of the helminth parasites of domesticated animals in India, came to the conclusion that these two species of the genus *Olveria* are very common in cattle and buffaloes. In the present communication the results of work on the life history of *Olveria indica* are recorded.

Material and Methods

The mature worms were collected from the cattle slaughter houses in Lucknow and were kept in Petri dishes in water in the laboratory at room temperature, when the eggs were laid and studied alive to note the early developmental stages at the time of laying. Some of the worms were teased in distilled water to obtain the eggs from the uterus. They were washed in water several times and when clear of all debris and organic matter were kept in distilled water at room temperature. Some of the eggs were also placed in the incubator at 90°–94°F. The water was changed twice a day, morning and evening, to prevent the growth of bacteria and fungi in the culture and normal development of the egg into miracidial stage took place. Examination of the larval stages was carried out usually in the living condition.

Laboratory bred snails belonging to different genera and species of snails were tested to ascertain the infectivity of the snails to miracidia and to notice their chemotactic attraction for the miracidia. Further, all the snails were kept under observation in clean water for several days till the cercarial forms were discharged from the molluscan hosts. The cercariae could be seen swimming when the glass beaker was held against the light and examined with a hand lens. When the cercariae were frequently discharged, they were examined microscopically and the snails were kept alive for further observations.

Earlier stages were obtained by dissection of these infected snails, the liver being the usual site for their development. The first rupture of the body results in a swarm of the characteristic organisms

into the surrounding water. Further search is made by squashing the whole molluscan body between the two slides and examining it under the low power of the microscope.

The cercariae were allowed to encyst on the lettuce leaves used for feeding the snails and these leaves with encysted metacercariae were fed to experimental animals for the recovery of the later stages.

Neutral red gave the best intra-vitam stain for the larval stages and Ehrlich's acid haematoxylin for the mature worms. The cercariae when treated with Schneider's aceto-carmine are cleared of their deep pigment that is dissolved by this reagent and the internal structures are stained to yield a clear picture of its anatomy.

The Egg

The eggs are available only at a certain time of the year. The specimens obtained from April onward yield a good supply of eggs and in June the maximum number were found. They were kept for incubation and in most of them miracidia developed.

The eggs are oval in shape and have a thin colourless operculate shell ; when deposited they are yellowish-brown in colour, due to the presence of yolk granules. The average size is 0·13 mm. by 0·015 mm. The egg contains a transparent somatic cell which is unsegmented at the time of laying. Within 24 hours of incubation, cleavage starts and 2–4 celled stage is observed (Fig. 1). Thereafter the segmentation proceeds rather quickly and within three days the embryo consists of an irregular mass of cells, inside the shell, measuring 1·5 mm. by 0·5 mm. in size. The embryo gradually increases in size and the yolk cells are used up (Fig. 2). Between the embryo and the shell a fluid substance appears and the embryo shows slow movements of contraction and expansion. On the eighth day of incubation differentiation of the embryo takes place. The body becomes covered by cilia and inside it a pair of eye spots, cephalic glands and germ balls are developed. The embryo becomes active and shows considerable movement. It strikes against the operculum with its head papilla to push its way out of the egg shell ; ultimately the operculum is thrown off and the miracidium emerges. During this period a wave-like current due to the constant beating of the cilia from the anterior to the posterior end of the body can be observed while the embryo is still inside the shell. In a healthy culture the miracidia emerge from the shell in 8–14 days. They usually come out, as in other forms, in the early hours of the morning between 6–10 a.m. and are seen actively swimming in water in a spiral motion and can be easily picked up in a dropper from the dish.

THE MIRACIDIUM

A mature miracidium is conical in appearance, with a broad anterior end bearing a head papilla and a narrow posterior end. It is 0·145–0·225 mm. in length and 0·054–0·068 mm. in breadth at about the middle of the body, narrowing towards either end. The body is covered with large epidermal plates (Fig. 4) arranged in four rows and bear a covering of cilia arising from these plates. The first row, the anterior, consists of six plates, the second row of eight plates, the third row of four plates and the last or fourth row of two plates only, all wedged together to form a complete armour. Their nuclei are situated towards the posterior end of each plate, except in the fourth row, where they are towards the anterior end. Thus, they represent the typical amphistome pattern. Below the epidermal plates is a layer of thin elongated oval cells constituting the sub-epithelial layer, that lines the central cavity of the miracidium. It multiplies actively to give rise to the "germinal cells" that migrate to the central cavity. Each germinal cell contains a prominent nucleus and granular cytoplasm. These germinal cells aggregate to form a *germ-ball* in the posterior third of the body. Another *germ-ball* is formed in the middle third of the body and each consists of 13–17 germ cells. Occasionally some of the germ cells break off from the germinal mass and float in the central cavity of the miracidium. Behind the anterior protrusible head papilla is a sac-like primitive *gut*, measuring 0·05 mm. in length. On either side of this gut are two elongated flask-shaped *cephalic* or *penetration glands* that appear to open at the base of the papilla. In the region of the gut are a pair of eye spots, beneath which is situated an irregular nerve mass.

There are two flame cells—one on either side—near the middle of the body (row 2–3 of the plates), somewhat close to the margin. These cells are pointing backwards with their ducts running forward as narrow tubes. Each turns backward as a coiled tube that opens posteriorly at the junction of the third and fourth row of the epidermal plates.

THE INTERMEDIATE HOST

A large number of snails belonging to different genera, *Limnaea*, *Indoplanorbis* and *Gyraulus*, were reared in the laboratory for experimental infection with the miracidia soon after they were hatched. When miracidia were introduced into the beakers containing the snails, they first moved towards them but then moved away from most of the snail species. In the case of *Gyraulus convexiusculus*, it was found that after some hesitation they aggregated round the foot of this snail and, from the subsequent emergence of cercariae from them, that they had penetrated into the interior.

Development inside the intermediate host proceeded normally and it was found that the first cercaria emerged from the infected snails after 30 days, but emergence after the 24th day was also observed. The cercariae continued to be shed even after 65 days of infection and even for a longer period afterwards.

The Sporocyst

The miracidium, soon after its penetration into the tissue of the snail, at first sheds its ciliated plates and cephalic glands. The flame cells, gut, eye spots and germinal cells are still to be seen.

On the fourth day of the infection a simple thin walled sac-like, oval body, 0·175 mm. by 0·118 mm. was recovered from the mantle cavity. It contained germinal cells packed in masses. Besides three such masses (solid), there were a few germinal cells also. In some sporocysts (Fig. 6) only two germ balls were seen and they had two flame cells one on either side. The germinal mass in a young sporocyst consists of 10–15 cells, each germ cell having a prominent nucleus. The sporocysts increase in size and may attain 0·4 mm. by 0·2 mm. after about seven days. The larger sporocysts contain, besides germinal cell masses, young developing rediae. Thus a 15 days old sporocyst, 0·4 mm. by 0·24 mm., contains five germ masses and two immature rediae, while after 33 days the sporocyst (0·45 × 0·235 mm.) contained seven germ balls at various stages of development, a few stray germ cells and two larvae showing a pharynx and a gut sac and thus resemble a redia (Fig. 7).

The wall of the sporocyst is thin consisting of a cuticle with a layer of muscle fibres and sub-epithelial cells having nuclei distributed over the surface of the sub-epithelium.

The Redia

The rediae are visible to the naked eye as small white specks. They are elongated bodies which contract and expand and measure 0·2–1·0 mm. in length and 0·07–0·2 mm. in breadth according to the degree of development. Germ balls are present in all the stages.

Abbreviations used in Text-Figures and Plate

ac. = acetabulum. **b.p.** = birth pore. **cil.** = cilia. **c.gl.** = cephalic glands. **c.** = cercaria. **e.** = eye spots. **ex.b.** = excretory bladder. **ex.d.** = excretory duct. **ex.p.** = excretory pore. **ex.pl.** = excretory plexus in the region of the intestinal caeca. **fl.c.** = flame cells. **g.b.** = germ balls. **g.c.** = germ cells. **g.w.** = germ wall. **int.** = intestine. **i.c.** = intestinal caeca. **l.e.d.** = longitudinal excretory duct. **m.** = mouth. **o.d.** = oral diverticulum. **oes.** = oesophagus. **o.s.** = oral sucker. **ov.** = ovary. **pap.** = oral papilla. **ph.** = pharynx. **pl.** = epidermal plates. **pig.** = radiating pigment. **red.** = redia. **s.** = intestinal sac. **t1, t2** = testes. **t.e.d.** = transverse excretory duct.

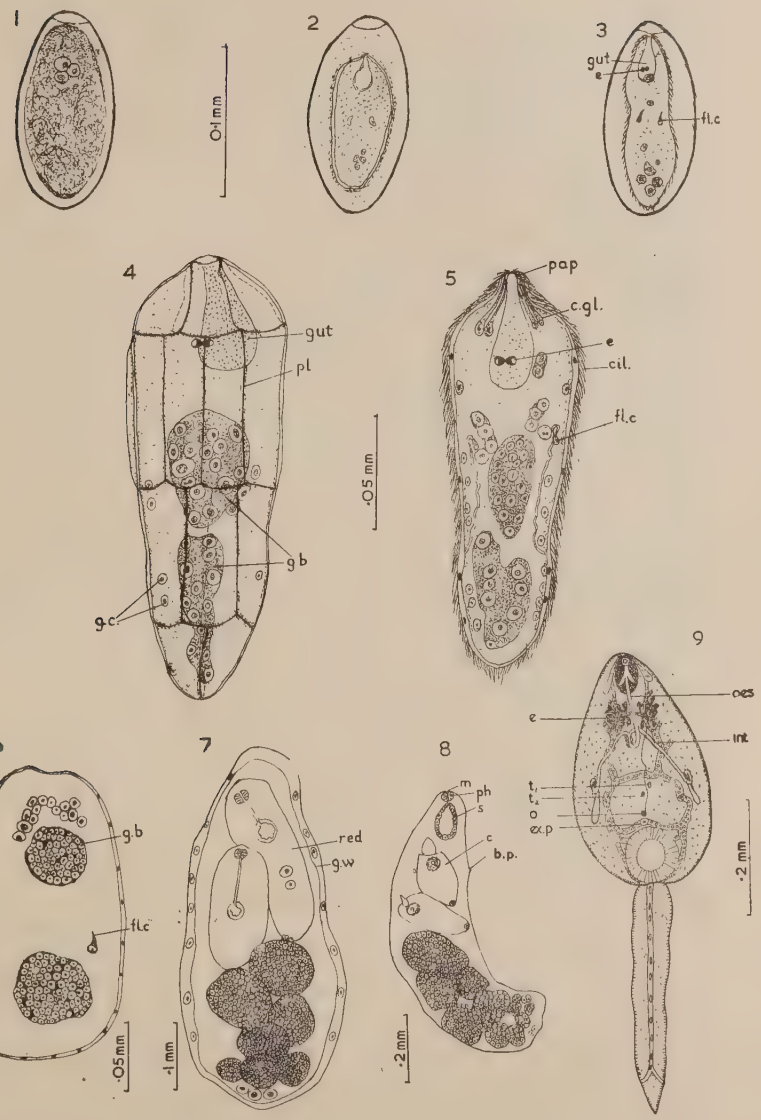


Fig. 1.—Segmenting egg of *Olveria indica*. Figs. 2 & 3.—Eggs of *O. indica* with developing embryos. Fig. 4.—Miracidium, showing epidermal plates. Fig. 5.—Miracidium, showing general morphology. Fig. 6.—Sporocyst recovered on the 4th day of infection. Fig. 7.—35 days old sporocyst showing developing rediae inside. Fig. 8.—Redia showing developing cercariae and germ balls. Fig. 9.—Cercaria, showing general morphology.

A redia which measures 0·6 mm. by 0·16 mm. has a muscular pharynx 0·04 mm. long and a sac-like gut 0·07 mm. long. It contains eight to fifteen germ balls, of different sizes. The cells lining the intestine are large with prominent nuclei. A mature redia measures 1 mm. in length and is 0·2 mm. in breadth at about the middle of the body. It has a small circular mouth followed by the muscular pharynx and sac-like intestine. The excretory system consists of three flame cells on each side—one at the base of the intestine, one near the posterior end and one at about the middle of its length. The excretory pore is about three-fourths the length from the anterior end, a little behind the middle pair of flame cells, opening on either side. There is no collar or lateral appendages (lappets), but at quarter the distance from the anterior end, there is a birth pore through which cercariae pass out (Fig. 8). I have not recovered any redia with daughter rediae inside it, but developing cercariae were observed, thereby indicating only one redial stage in the life history of this form. The mature rediae contain well differentiated cercariae apparently ready to escape.

The Cercaria

The cercariae are liberated from the rediae and complete their development outside the body of the redia. They are easily visible to the naked eye, owing to the presence of deep pigment in the body, as minute specks near the water surface and are extremely contractile. The period of development of cercariae varies from 24 to 43 days, but sometimes it takes as many as 70 days.

The following table indicates the data for the calculation of cercarial development from the miracidia :—

Date of infection	Date of emergence of cercariae	Time taken
12.5.1952	6.6.1952	26 days
3.6.1952	9.7.1952	38 days
2.8.1952	25.8.1952	24 days
3.8.1952	15.9.1952	43 days
3.9.1952	15.11.1952	74 days

Generally the cercariae emerge in the early hours of the morning, but their free swimming life is not very long and they soon begin to encyst on the vegetation kept in the beaker containing experimental snails (Plate I, Fig. 1). Some of them were observed in the encysted condition near the water surface on the wall of the containers.

The body of the cercaria is pear-shaped and it is extremely contractile. It measures 0·41 mm. in length and 0·21 mm. in maximum breadth and the tail is 0·36 mm. long and 0·05 mm. broad in

fixed specimens. It is covered with thick cuticle and is heavily pigmented, so that the internal anatomy is not easily studied in the living condition. The pigmentation starts round the eyes near the anterior end and forms long radiating strings extending over a large area on both sides. The eyes are conical structures with a clear lens. In addition there are cystogenous cells containing rod-like granules which make the entire body opaque and obliterate most of the internal structures. When treated with Schneider's aceto-carmine the pigment is dissolved and the various structures are stained and become visible. The acetabulum at the posterior extremity is large and round, about 0·09 mm. in diameter. The oral sucker is smaller being 0·05 mm. broad and 0·06 mm. long and bears two diverticula embedded in it. The oesophagus is 0·11 mm. long and is curved forward at the posterior end. It divides into two coiled intestinal diverticula extending backwards to about 0·05 mm. in front of the acetabulum. The rudiments of the genitalia are also present, consisting of a small rounded ovary in front of the excretory vesicle and a string of cells running forward from it indicating its duct. Rudiments of two testes, one in front of the other, are also seen in front of the ovary.

The excretory system is seen in younger specimens of cercariae prior to the formation of the pigment and cystogenous cells. It consists of a rounded vesicle immediately in front of the acetabulum with a small opening on its dorsal side. Laterally two longitudinal ducts arise, one on either side; these run forward in the body and at about the middle of the body length slightly bend inward giving off a transverse duct running medially to meet its fellow of the other side; they then run forward and outward up to the eyespots where each divides into three narrower ducts. In the course of their length the longitudinal ducts give off finer branches some of which in the young and mature flukes appear to form a network or plexus surrounding the intestinal diverticula, as already reported by Thapar and Sinha (1945) in the adult worms.

The caudal excretory duct arises from the posterior end of the excretory vesicle and passes back through the tail to terminate near its posterior extremity.

The main excretory ducts are full of refractile granules of various sizes.

ENCYSTMENT

As stated earlier, the free life of a cercaria is of short duration, never more than 20–25 minutes, when it begins to encyst by attaching itself to vegetation or to the wall of the container near the water surface. When about to encyst, it stops active movements of the

body, attaches itself by its oral sucker to the vegetation, moving its tail to and fro. The cystogenous cells are extruded and are distributed over the surface evenly to form a covering round the body of the cercaria. When the tail is cast off the cysts are rounded and brown in colour. They are easily visible to the naked eye being 0·2 mm. in diameter. When examined under the high power of the microscope they appear to consist of two layers—an outer thick brown one and an inner thin transparent layer—inside of which is the body of the metacercaria. Inside the body refractile excretory granules and some pigmentation are visible.

Experimental Infection

Only one buffalo calf was available for infection and it was kept and fed in the laboratory since birth, feeding on milk and later on sterile vegetable leaves. When it was accustomed to vegetable feeding, metacercariae encysted on the lettuce leaves were given to the calf for about three weeks, an average of about 200 cysts daily. The last batch of metacercarial cysts was administered to the calf on the 15th of November 1952 and the calf was kept under observation. After some time it showed signs of general debility and emaciation and occasionally passed loose faeces, but the faecal examination revealed no eggs or larvae of any kind. Ultimately it was sacrificed and autopsied on the 15th of February 1953. The results of the post mortem examination revealed the presence of amphistomes, all immature, which resembled *Olveria indica* in all essential features except that the sexual organs were not fully mature and the eggs were not developed. A thorough search of the contents of the rumen yielded a total of 40 immature specimens. The general structure of an immature worm after treatment with KOH solution is represented in Plate I, Fig. 2.

Summary

The life history of *Olveria indica* was worked out experimentally at Lucknow. *Gyraulus convexiusculus* was shown to be the intermediate host. The various stages in the larval development are described in detail.

References

Tandon, R. S., 1951.—"On a new amphistome, *Olveria bosi* n.sp. from the rumen of buffalo, *Bos bubalis*, from Lucknow." *Indian J. Helminth.*, **3**, 93–100. (W.L. 9939g).

Thapar, G. S., 1956.—"Systematic survey of helminth parasites of domesticated animals in India." *Indian J. vet. Sci.*, **26**, 211–272. (W.L. 9941h).

———, and Sinha, B. B., 1945.—"On the morphology of a new genus of amphistomes from the rumen of cattle in the United Provinces." *Ibidem*, **15**, 219–222.

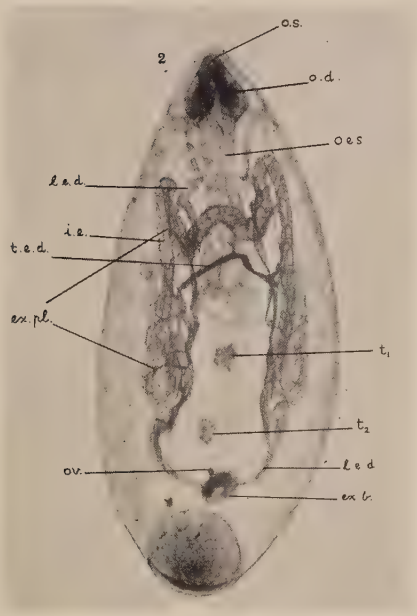


PLATE I

Fig. 1.—Metacercariae encysted on lettuce leaf. Fig. 2.—Photomicrograph of an immature *Olveria indica* treated with KOH to show excretory system and its intestinal plexus.

(Fig. 2 is reproduced by courtesy of the *Indian J. vet. Sci.*)

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 187–190.

Sur un Curieux Nématode, *Robertia leiperi* gen. et sp. nov., Parasite de l'Intestin Postérieur de Diplopode

Par L. Travassos et G. R. Kloss
Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Les nématodes des diplopodes malgré les recherches de Leidy depuis plus d'un siècle, ont été peu étudiés par les auteurs plus récents. Au Brésil, la faune de diplopodes est très riche, et les espèces de ces arthropodes récemment décrites par le Dr. Otto Schubart sont nombreuses. Accompagnant la richesse des espèces donnant l'hospitalité, leurs espèces parasites sont considérablement nombreuses. Nos études nous ont amené à admettre une grande spécificité entre parasite et hôte. Certaines espèces de nématodes ont été référées des "hôtes" d'espèces très éloignés systématiquement et avec une ample distribution géographique, et il nous semble que ce fait soit dû aux difficultés qu'il y a de les caractériser convenablement.

Un facteur qui certainement contribue à une connaissance moins parfaite de ces parasites, est constituée par la difficulté d'obtenir du matériel bien fixé et en bonnes conditions d'étude ; dans quelques groupes la difficulté d'obtenir des exemplaires du sexe masculin complique également sa caractérisation parfaite. Il est à noter que l'emploi de solution saline habituellement utilisée lors de la collecte de matériel de vertébrés, pour lavage, ne convient pas aux nématodes d'arthropodes ; ceux-ci demandent une solution bien plus concentrée ($16^0/_{00}$). Pour les nématodes, la fixation rapide en fixateur fortement chauffée (90° à 100°) et qui soit aussi isotonique, est également indispensable. L'emploi de colorants faibles et de coloration lente (carmin acétique dilué) permet de bonnes colorations qui dans le matériel diaphanisé au phenol et créosote de hêtre mettent en évidence non seulement les détails cellulaires mais encore les structures chitineuses.

Des études plus récentes concernant les nématodes de Diplopodes ont permis de les séparer en différentes familles nettement distinctes. A côté du groupe important des Thelastomatidae dans lequel on doit inclure les espèces dont les mâles sont toujours rares et de taille réduite, nous pouvons rencontrer diverses autres familles bien caractérisées par la structure céphalique et par l'appareil reproducteur.

A côté de la grande famille Thelastomatidae, avec différentes sous-familles qui parasitent outre les Diplopodes, divers autres groupes d'arthropodes, en particulier *Blattaria*, nous considérons différentes autres familles exclusives de Diplopoda, telles que Ichthyocephalidae, Rhigonematidae, Carnoyidae, etc. Les parasites que nous étudions plus loin se rapprochent des Thelastomatidae par la structure de l'oesophage ; toutefois, leur structure, très simplifiée en raison de sa grandeur réduite, ne permet pas un rapprochemente à n'importe lequel des groupes dans lesquels cette grande famille vient d'être récemment divisée. Nous proposons une sousfamille nouvelle jusqu'à ce que d'autres espèces voisines permettent de bien localiser le genre dans la famille Thelastomatidae ou dans une autre indépendente.

Robertiinae subfam. nov.

Thelastomatidae à corps très réduit et organes reproducteurs féminins situés dans la partie postérieure du corps. Moitié antérieure très mince, occupeé exclusivement par le petit oesophage et l'intestin. Appareil reproducteur monodelphe. Mâle plus petit que la femelle, avec spicule très reduit et unique et l'extremité posterieur sous-conique. Ouverture génitale féminine à la moitié postérieure du corps qui se termine en forme arrondie, présentant un filament très mince.

Genre type et unique—*Robertia* gen. nov.

Robertia gen. nov.

Robertiinae. Avec les caractéristiques de la sous-famille. Espèce-type et unique—*R. leiperi* sp. nov.

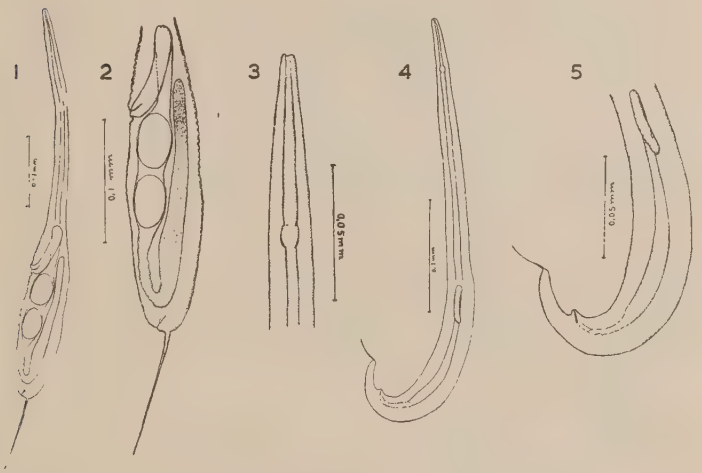
Robertia leiperi sp. nov.
(Figs. 1–5)

Longueur : Mâle 0·44 mm ; femelle 0·63 à 0·76 mm.

Largeur maxima : Mâle 0·028 mm.; femelle 0·050 à 0·076 mm.

Mâle claviforme, avec la partie antérieure du corps très allongée et occupée seulement par le tube digestif. L'appareil reproducteur occupe à peine le tiers postérieur du corps. L'extrémité caudale est courbée en arc et se termine par un filament relativement long, cuticulaire. La cuticule est finement striée transversalement. Extrémité

antérieure très mince sans laisser entrevoir de vestiges de lèvres ni de papilles. Anneau nerveux et pore excréteur non-observés. L'oesophage se trouve à la suite d'un "stoma" très mince et relativement long. Il présente un *corpus* étroit et cylindrique qui se termine postérieurement dans un bulbe rudimentaire où l'on n'observe pas de valves chitineuses. Intestin sub-rectiligne et cylindrique, s'élargissant suavement vers la partie postérieure. L'ouverture ano-génitale



Robertia leiperi gen. et sp. nov.

Fig. 1.—Femelle total ; Fig. 4.—Mâle, total ; Fig. 3.—Extremité antérieure du mâle ; Fig. 2.—Extremité postérieure, femelle ; Fig. 5.—Extrémité postérieure du mâle.

est située dans une saillie prononcée du corps. La queue est initialement conique et se termine par un filament cuticulaire presque aussi long que la partie conique. Il n'existe pas d'ailes caudales et il n'a pas été observé non plus de papilles. L'appareil reproducteur est constitué par un tube sub-rectiligne courbé à l'extrémite distale qui constitue la partie glandulaire. Il semble exister un rudiment de spicule. L'oesophage a environ 0·06 mm. de longueur et l'ouverture cloaquale se trouve à près de 0·04 mm. de l'extrémité caudale (y compris le filament terminal). Le filament terminal à environ 0·016 mm.

La femelle a le tiers postérieur du corps beaucoup plus dilaté que la partie antérieure qui est près de $\frac{1}{3}$ plus mince. La cuticle est fortement striée dans la partie dilatée, et les stries deviennent progressivement plus étroites jusqu'à disparaître dans la partie anterieure. L'extrémité antérieure présente une ouverture buccale simple suivie d'un "stoma" très mince et relativement long. L'oesophage est comme celui des mâles. L'anneau nerveux ni le pore excréteur n'ont pas été observés. Le tube intestinal est comme celui des mâles, sub rectiligne et cylindrique et se termine par un court rectum près de la base du filament cuticulaire. L'extrémité postérieure est arrondie ayant un long filament cuticulaire terminal. L'appareil reproducteur est monodelphe, l'ouverture externe étant située dans la portion plus antérieure de la partie dilatée du corps. Ovejecteur dirigé vers l'extrémité antérieure, prenant aussitôt la direction postérieure et se transformant en uterus. Celui-ci contient, au maximum, deux grands oeufs. Oviducte se dirigeant initialement en arrière, pour se courber aussitôt en V et se transformer en ovaire rectiligne de direction antérieure qui se termine au niveau du pore génital. L'oesophage a une longueur totale au près de 0·068 à 0·140 mm., sur une largeur de 0·006 à 0·012 mm. L'anus est à près de 0·093 à 0·104 mm. de l'extrémité du filament caudal. L'ouverture génitale est à près de 0·24 à 0·26 mm. de l'extrémité postérieure. Les oeufs mesurent près de 0·048 à 0·056 mm. de long sur 0·028 à 0·032 mm. de largeur ; la coque est relativement mince.

Habitat : Intestin postérieur de *Eurydesmus ruidus* Verhoeff.

Provenance : Paineiras (Corcovado), Rio de Janeiro, D. F. Brésil.

Holotypus femelle et *allotypus* mâle dans la Collection Helminthologique de l'Institut Oswaldo Cruz sous le n° 24.378 ; 9 paratypus sous les n° 24.379 a 24.382.

Nous avons examinés 10 exemplaires femelles et 1 mâle de cette espèce.

Bibliographie

Dollfus, R. Ph., 1952.—"Quelques Oxyuroidea de Myriapodes." *Ann. Parasit. hum. comp.*, **28**, 143–236. (W.L. 899a).

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 191–198.

Über eine unsegmentierte, eingeschlechtliche *Taenia saginata*

Von H. Vogel

Aus der Helminthologischen Abteilung des Bernhard-Nocht-Instituts für Schiffs- und Tropenkrankheiten, Hamburg

Bildungsanomalien verschiedener Art kommen bei Cestoden relativ häufig vor. Wiederholt wurden dreikantige Taenien mit Y-förmigem Querschnitt und einem Scolex mit 6 Saugnäpfen beschrieben, Zwischenschaltung unvollständiger keilförmiger Glieder, gefensterte Proglottiden, gegabelte Gliederketten und Umkehrung oder Verdopplung des Genitalapparates. Eine weitere Missbildung besteht darin, dass Glieder miteinander verschmolzen sind, so dass auf mehr oder weniger langer Strecke Gliedergrenzen zwischen den aufeinanderfolgenden Genitalsätzen fehlen. Diese Anomalie wurde von Colin (1875) als "*Taenia fusa*" oder "*T. continua*" bezeichnet. In den meisten veröffentlichten Fällen dieser Art war das Fehlen der Segmentation auf einzelne Stücke eines sonst normal gegliederten Wurmkörpers beschränkt. Hierher gehören z.B. folgende bei *Taenia*-Arten des Menschen beobachtete Fälle: ein Sammlungsexemplar von 1830 des Royal College of Surgeons in London, das Blanchard (1894) beschrieb und abbildete, die Fälle von Colin (1875), Vallin (1877), Welch (zit. n. Blanchard 1889), Bork (1891) und eine Eigenbeobachtung von Blanchard (1894). Auch bei dem Hundebandwurm *Taenia pisiformis* sind entsprechende Missbildungen von Teilen der Strobila beschrieben worden (Neumann, 1896, Roudabush, 1941).

Ein Fehlen der Segmentation im ganzen Taenienkörper scheint hingegen sehr selten zu sein. Es lag wahrscheinlich bei der von

Hussey, Shookhoff und Sterman (1952) bei einer Mexikanerin beobachteten Taenie vor, die zwar nicht als ganzer Bandwurm gewonnen werden konnte, aber doch immerhin in Stücken von zusammen ungefähr $1\frac{1}{4}$ m. Länge. Diese Teile liessen jede Gliederung vermissen. Auch bei einer von Cobbold (1884) erwähnten, leider nicht näher beschriebenen *Taenia saginata*, die er von Prof. Aitken als vollständigen Bandwurm, jedoch ohne Kopf, erhalten hatte, scheint sich der Mangel einer Segmentation auf den ganzen Wurm erstreckt zu haben.

Die im folgenden beschriebene missgebildete Taenie gehört ebenfalls zu den in ganzer Länge ungegliederten Exemplaren. Sie verdient eine Mitteilung noch deshalb, weil ein kompletter Bandwurm samt Scolex vorliegt, der eine eindeutige Artbestimmung erlaubt, und weil die Untersuchung der Genitalorgane ergeben hat, dass zusammen mit der Gliederung auch die Ausbildung weiblicher Fortpflanzungsorgane unterdrückt worden ist.

Herkunft und Verarbeitung des Materials: Eine 47-jährige, am Stadtrand von Hamburg wohnende Hausfrau bemerkte im August 1955, dass mit ihrem Stuhl von Zeit zu Zeit bandförmige helle Stücke abgingen. Ein derartiges Gebilde, das uns Herr Dr. Stockenius in Form histologischer Schnitte vorlegte, liess infolge starker Mazeration keine sichere Deutung zu. Die Patientin wurde veranlasst, neues Material zu liefern, und brachte am 5. Oktober in physiologischer Kochsalzlösung ein weiteres frisch abgegangenes Stück. Das bandförmige Gebilde, das 17 cm lang, etwa 4 mm breit und von hellbrauner Farbe war, liess keinerlei Gliederung erkennen. Von den beiden Enden quer zur Längsrichtung angefertigte Paraffinschnitte zeigten die Strukturen eines Bandwurmes, ein lockeres Parenchym mit vorwiegend längs verlaufenden Muskelfasern, das eine Trennung in eine Mark- und eine Rindenschicht erkennen liess, zahlreiche konzentrisch geschichtete Kalkkörperchen, seitliche Exkretionskanäle und leere Schläuche im Mittelteil. Am 9. Oktober erhielten wir nochmals ein 3 cm langes ungegliedertes Wurmstück mit unregelmässig zerfetzten Enden.

Da ohne Zweifel ein ungewöhnlicher Bandwurm vorlag, veranlassten wir die Patientin, die Klinik des Bernhard-Nocht-Instituts (Chefarzt Professor Dr. Mohr) zur Durchführung einer Bandwurmkur aufzusuchen. Die körperliche Untersuchung ergab keinen krankhaften Befund. Auch das Blutbild zeigte nichts Besonderes. Am 15. Oktober wurde eine Kur mit 8 g Extractum

filicis durchgeführt mit dem Erfolg, dass der Wurm samt Scolex abging.

20 Stücke des Wurmes von 2, 5—4 cm. Länge, die in verschiedener Höhe seines Körpers entnommen worden waren, wurden zu Totalpräparaten verarbeitet und mit Milchsäure-Karmin gefärbt, teils vor, teils nach der Fixierung in 70%igem Alkohol. Von einigen Wurmstücken wurden auch Paraffinschnitte quer zur Längsrichtung angefertigt.

Morphologische Befunde: Bei der Abtreibung war das vorderste Achtel des Wurmes in 4 Stücke zerrissen, die sich zusammen mit dem als einheitliches Band abgegangenen Restkörper zu einem vollständigen Wurm zusammenfügen liessen. Dabei ergab sich eine Gesamtlänge des erschlafften, noch unfixierten Cestoden von 3,67 m. Der Scolex, der sich in Zusammenhang mit einem 2,5 cm. langen Stück des Wurmkörpers befindet, ist unbewaffnet und 1,4 mm. breit. Auf der Scheitelmitte befindet sich ein Grübchen und unmittelbar unter diesem ein kleiner napfförmiger Muskelapparat. Dieser gleicht ganz der von Leuckart (1886) bei *Taenia saginata* beschriebenen und abgebildeten Struktur, die einem Rostrum homolog ist. Die 4 Saugnäpfe haben einen grössten Durchmesser von 0,49 mm. und einem kleinsten von 0,43 mm. Der Wurmkörper weicht von dem gewohnten Bilde einer Cestodenstrobila in auffälliger Weise darin ab, dass er—einer flachen Nudel ähnlich—ein einheitliches, von keinen Gliedergrenzen unterbrochenes Band bildet, dass er also keine Kette ist sondern im wahrsten Sinne des Wortes ein "Bandwurm". Seine grösste Breite von 8–9 mm. erreicht der Wurm zu Beginn des letzten Viertels und verjüngt sich dann bis zum Hinterende auf etwa 5 mm. In seinen breiteren Abschnitten sind die Seitenränder leicht gekräuselt, und auf den beiden Körperflächen sind drei längs verlaufende flache Rillen angedeutet, von denen eine in der Mittellinie und zwei über den Exkretionskanälen verlaufen. Papillenartige Erhebungen, die Hussey und Mitarbeiter auf den beiden Flächen bei ihrem Exemplar feststellten, sind nicht vorhanden, auch erheben sich die Lippen der Genitalatrien, soweit solche vorhanden sind, seitlich nur wenig über die Ränder des Bandes. Über die Ober- und Unterfläche ziehen feine parallele Falten, in der Regel schräg zur Längsrichtung. Auf einer kurzen Strecke des vorderen Wurmabschnittes scheinen einige mehr oder weniger regelmässig angeordnete Querrillen oder Falten eine äussere Segmentierung anzudeuten. Wie die nähere Betrachtung zeigt, handelt es sich jedoch nicht um eine echte Gliederung, und die seitlichen

Hodenfelder erfahren an diesen Stellen nicht die scharfe Unterbrechung, die man bei normalen Rinderbandwürmern an den Proglottidengrenzen feststellen kann.

An der Muskulatur fallen keine merklichen Abweichungen vom Normalen auf. Vom Exkretionssystem ist jederseits ein weitlumiger Längskanal zu sehen, der als der ventrale Exkretionskanal angesprochen werden muss. Die Querkommissuren, die dieses Kanalpaar normalerweise an den Hinterenden der Proglottiden verbinden, fehlen in der ganzen Länge des Wurmes, auch in dem erwähnten Stück, wo durch Falten eine Segmentierung vorgetäuscht wird. Im vordersten Abschnitt des Wurmes sind neben den ventralen auch dorsale Exkretionskenäle zu erkennen.

Von den Fortpflanzungsorganen sind die Testes am besten ausgebildet und, abgesehen vom vordersten Abschnitt, im ganzen Wurm bis zum Hinterende vorhanden. Sie sind innerhalb der beiden Exkretionskanäle in zwei seitlichen Streifen angeordnet, die sich ohne Unterbrechung durch den ganzen Körper fortsetzen. Im vorderen Teil des Wurmes reichen die Hodenfollikel bis zur Mittellinie, während sie in den hinteren Wurmabschnitten einen medianen Streifen frei lassen. Die Vasa efferentia sind an vielen Stellen gut zu erkennen. Sie laufen auf hintereinandergereihte Zentren zu, in denen sie sich zum Vas deferens vereinigen, wo ein solches vorhanden ist. Hinsichtlich der Vollkommenheit, mit der die Ausführgänge des männlichen Geschlechtsapparates ausgebildet sind, lassen sich drei Grade unterscheiden: 1. Es sind keinerlei Ausführgänge oder nur Vasa efferentia vorhanden, die häufig unregelmässig geformte Auftreibungen zeigen (Figur 1, oben und unten). 2. Die Vasa efferentia treten zu einem verkümmerten Vas deferens zusammen, das die Randpartie des Körpers nicht erreicht. Das Gefäss bildet in der Körpermitte infolge angestautrer Geschlechtsprodukte einen rundlichen oder unregelmässig gestalteten Sack, an den sich evtl. noch ein gewundener Schlauch anschliesst, der blind endet. Cirrusbeutel und Genitalatrium fehlen in der entsprechenden Randpartie. 3. Das Vas deferens ist normal ausgebildet. Es steht mit einem Cirrusbeutel in Verbindung und dieser mit einem Genitalvorhof, der in unregelmässigem Wechsel am linken oder rechten Körperrande sitzt. Figur 2 zeigt eine Wurmstrecke, auf der die männlichen Leitungswege alle bis auf den obersten Genitalsatz wohlentwickelt sind. In manchen anderen Abschnitten des Wurmkörpers herrschen die Formen 1 und 2 mit verkümmerten Ausführgängen vor, und es lassen sich dort auch auf längeren Strecken

Figur 1.—Stück aus dem mittleren Teil der ungegliederten Taenia saginata. Die Testes sind in einem ununterbrochenen Band angeordnet. Ihre Vasa efferentia vereinigen sich nur im mittleren Bildteil zu einem Vas deferens. Figur 2.—Die Ausführgänge der männlichen Sexualorgane sind in diesem Abschnitt der hinteren Körperhälfte fast überall gut entwickelt. Stellenweise sind die Uterusäste (doppelt konturiert) zu sehen. Figur 3.—Abschnitt aus der hinteren Wurmhälfte mit atypisch gestalteten Uteri. In 3 von den 6 erkennbaren Genitalsätzen fehlen die männlichen Ausführgänge. (Massstab wie in Figur 2).

keine Genitalatrien nachweisen. Die Verteilung der drei Typen im Wurmkörper ist ganz unregelmässig und nicht von dem Alter des Körperabschnittes abhängig.

Eine weitere Besonderheit dieses Wurmes liegt darin, dass in seiner ganzen Länge nirgends Ovarien, Vaginae, Receptacula seminis und Schalendrüsen vorhanden sind. Auch Dotterstöcke sind nicht mit Sicherheit auszumachen, es sei denn, dass man spärliche Gruppen bräunlicher Zellen, die hier und da zwischen den Vasa efferentia liegen, als Rudimente eines Dotterstockes ansprechen will. Das einzige Organ des weiblichen Genitalapparates, das sich eindeutig erkennen lässt, ist der Uterus. Erwartungsgemäss sind die Uteri am besten in den hinteren Partien des Wurmes ausgebildet. Wie Figur 3 und stellenweise auch Figur 2 erkennen lassen, weichen die Uterus-Figuren stark von dem üblichen Bau des *Taenia*-Uterus ab. Die Äste sind unregelmässig verzweigt. Ihre Zahl ist viel geringer als bei einer normalen *Taenia saginata*. Meistens fehlt ein medianer Längsstamm oder ist sehr kurz. In der Regel laufen die Uterus-Äste sternförmig auf ein Zentrum zu, das ungefähr mit der Ursprungsstelle des Vas deferens zusammenfällt, wo ein solches angelegt ist. Uteri können auch dort vorhanden sein, wo männliche Ausführgänge fehlen, z. B. an drei Stellen in Figur 3. Die Uterusschläuche enthalten weder Eier noch Ei- oder Dotterzellen. Sie sind grösstenteils leer und schliessen nur hie und da bräunliche Konkremente ein in Form lockerer Massen oder dichter Ballen. Die Natur dieser Einlagerungen konnte ebensowenig geklärt werden wie die anderer Klumpen, die im Parenchym in dem von den Exkretionskanälen eingeschlossenen Raume liegen und sich mit Milchsäure-Karmin dunkel färben (Figur 3).

Besprechung der Befunde: Der vorliegende Bandwurm gehört ohne Zweifel zur Gattung *Taenia*. Die Genitalorgane, besonders der Uterus, sind so stark missgebildet, dass sie für eine Artdiagnose unbrauchbar sind. Der in diesem Falle glücklicherweise vorliegende Scolex erlaubt es jedoch, den Wurm mit ausreichender Sicherheit als *Taenia saginata* zu bestimmen.

Bei dem vorliegenden Exemplar sind im wesentlichen zwei Anomalien miteinander kombiniert, ein totaler Verlust der äusseren Segmentierung und eine Unterdrückung des weiblichen Geschlechts. Der Mangel einer Gliederabgrenzung erstreckt sich teilweise auch auf innere Organe; denn es fehlen überall die Querverbindungen der

Exkretionskanäle, und die Hodenfelder bilden jederseits ein ununterbrochenes Band. Eine innere Metamerie kommt jedoch in den hintereinander angeordneten Uteri und Ausführgängen der Testes zum Ausdruck. Da einerseits die Hoden und teilweise auch deren Ausführgänge gut entwickelt sind, und andererseits alle Teile der weiblichen Genitalorgane, abgesehen von Uterus, fehlen insbesondere Ovarium, Vagina, Dotterstock und Mehlissche Drüse, so muss dieser Wurm als ein eingeschlechtlich-männliches Exemplar angesehen werden. Aus den älteren Veröffentlichungen über Fälle von *"Taenia continua"* des Menschen, in denen Angaben über die Fortpflanzungsorgane gemacht worden sind, geht hervor, dass die weiblichen Organe nicht unterdrückt waren. Bei dem Exemplar von Welch befanden sich Eier im Uterus und war eine Vagina vorhanden. Auch Bork (1891) erwähnt die Existenz einer Vagina, und Blanchard (1894) spricht von einem gut ausgebildeten Geschlechtsapparat. In diesen drei Fällen handelt es sich um Taenien, bei denen die Segmentierung nur streckenweise fehlte. Es ist bemerkenswert, dass das einzige, dem Verfasser bekannte Taenien-Exemplar mit totalem Verlust der Segmentierung, dessen Genitalorgane beschrieben worden sind, nämlich das von Hussey und Mitarbeitern (1952), gut entwickelte Testes, aber keine Ovarien aufwies, ganz entsprechend der hier beschriebenen Taenie.

Wenn man sich fragt, wie solche Anomalien zustandekommen können, die den Wurm in ganzer Länge betreffen, so wird man in erster Linie an eine Verlustmutation denken. Dass der Mangel einer Gliederung sowohl bei der vorliegenden Taenie wie bei der der amerikanischen Autoren mit dem Verlust der weiblichen Fortpflanzungsorgane verbunden war, ist möglicherweise kein Zufall. Es ist vorstellbar, dass die Gene, die für die Segmentation und für die Ausbildung weiblicher Geschlechtsdrüsen und -wege verantwortlich sind, in den Chromosomen so dicht beieinanderliegen, dass sie gemeinsam in Verlust geraten können. Die Ausbildung des Uterus hat sich im vorliegenden Falle und in dem von Hussey und Mitarbeitern als unabhängig von der Entwicklung der anderen weiblichen Fortpflanzungsorgane erwiesen.

Zusammenfassung: Es wird eine seltene Missbildung bei *Taenia saginata* beschrieben. Der Wurmkörper lässt in ganzer Länge die äusseren Gliedergrenzen und die Querverbindungen der Exkretionskanäle vermissen. Während die Testes und teilweise auch deren Ausführgänge gut entwickelt sind, fehlen alle weiblichen Organe, abgesehen vom Uterus. Wahrscheinlich liegt eine Verlustmutation vor.

Herrn Professor R. T. Leiper in Verehrung zu seinem 80. Geburtstag gewidmet, verbunden mit den besten Wünschen des Hamburger Tropeninstituts.

LITERATUR

BLANCHARD, R., 1889.—Traité de Zoologie Médicale. Vol. I. Paris. 808 pp.

————, 1894.—"Sur quelques cestodes monstrueux." *Progr. méd., Paris*, **20,** 1–17. (W.L. 17035.)

BORK, G., 1891.—"Über die Misbildungen bei Taenien." *Inaug. Diss.* Kiel.

COBBOLD, T. S., 1884.—"Description of *Ligula mansoni*, a new human cestode." *J. Linn. Soc.* (*Zool.*), **17,** 78–83. (W.L. 11296.)

COLIN, L., 1875.—"*Taenia fusa* ou *continua*." *Bull. Soc. méd. Hop., Paris*, **12,** 323. (W.L. 5281.)

HUSSEY, K. L., SHOOKHOFF, H. B. and STERMAN, M. M., 1952.—"An anomalous tapeworm from man." *J. Parasit.*, **38,** 305–307. (W.L. 11428.)

LEUCKART, R., 1886.—Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Vol. I. Leipzig and Heidelberg. 1000 pp.

NEUMANN, G., 1896.—"Notes sur des téniadés du chien et du chat." *Mém. Soc. zool.*, **9,** 171–184. (W.L. 13343.)

ROUDABUSH, R. L., 1941.—"Abnormalities in *Taenia pisiformis*." *Trans. Amer. Micr. Soc.*, **60,** 371–374. (W.L. 21400.)

VALLIN, E., 1877.—*Bull. Soc. méd, hôpitaux* **14,** 238–240.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 199–204.

The Breeding of *Bulinus truncatus* in Total Darkness

By J. M. Watson, D.Sc., A.R.C.Sc.

Director, Commonwealth Bureau of Helminthology, St. Albans, Herts. Formerly, Head of the Department of Zoology and Microbiology, College of Science, University of Baghdad, Iraq

and

A. S. Al-Ali, B.Sc.

Demonstrator, Department of Zoology and Microbiology, College of Science, University of Baghdad, Iraq

It has been remarked by all those who have had occasion to observe this snail that *Bulinus truncatus*, the molluscan intermediate host of *Schistosoma haematobium*, tends in general to avoid both densely shaded and completely exposed habitats in nature. In the laboratory it moves away both from bright light and from darkness (Barlow, 1937 ; Zakaria, 1955 ; Watson, 1956) ; and it has long been the consensus of opinion among malacologists and epidemiologists concerned with the control of schistosomiasis that the molluscan intermediate hosts of the human schistosomes are unable to establish themselves and breed in the complete absence of light. That this view has some basis in fact in the case of *Bulinus truncatus* has recently been confirmed by Deschiens and Bijan (1956) and Deschiens (1957), who found that, as compared with control populations in lighted aquaria, populations of this snail kept in the dark were reduced in number, oviposition was rare, eggs frequently failed to develop, and hatching juveniles showed a high mortality rate. Covering irrigation channels to exclude light has therefore been recommended as a control measure.

Nevertheless, colonies of *B. truncatus* breeding in darkness have been reported from time to time. Barlow *et al.* (1942–1951) found this species maintaining itself in cemented and covered canals in Egypt ; Deschiens (1952) recorded it as multiplying in darkness in wells in Mauritania ; while Marill (1943) discovered breeding colonies in closed canals in Algeria.

These findings stimulated the authors to attempt to maintain and breed this snail in total and continuous darkness in the laboratory.

Experiments and Findings

Egg-clutches of *B. truncatus* that had been deposited on water-plants and decaying fragments of vegetable matter were collected on 12.12.56 from the Abdul-Latif Al-Farras Canal at Tarmiyah, about 40 km. north of Baghdad, at a point some 12 km. west of its off-take from the west bank of the River Tigris. The temperature of the water at this time was only 6°C., owing to a spell of exceptionally cold weather ; but a few days previously it had been 11°C. to 12°C.

The egg-clutches were brought back to the laboratory and placed in a glass tank 17 cm. long by 11·5 cm. wide, filled with decanted Tigris River water to a depth of 7·5 cm. The tank was maintained at a temperature of 25°C. to 27°C in a closed incubator into which no light penetrated except for a period of less than two minutes, at first twice a week and later daily, when the incubator door was opened to allow rapid inspection and partial change of water, 500 ml. being removed and replaced by fresh river-water on each occasion.

The eggs had all hatched by 24.12.56, and since no algae were present on the sides of the tank to form a source of food (Standen, 1949), the infant snails presumably fed on the small quantity of decaying vegetable debris which formed a thin deposit at the bottom. Commencing two weeks after hatching, a small quantity of strained spinach (approximately 2 gm.) was added to the tank once weekly as food.

The young snails grew steadily, and by the beginning of March the survivors were four individuals, each with an extended foot length of 7·5 mm., and with a shell spire height of 8 mm. in two cases and 9 mm. in the other two. Later, by 28.4.57, the shell spire height had reached 11 mm. in one of these snails, 9 mm. in two others, and 8·5 mm. in the remaining individual. The factors causing such a difference in growth rates among these snails, living under apparently the same conditions, were not determined by the authors.

Mortality among the infant snails was very high. Thus, out of a total hatch of 200, only four had survived. Unfortunately, in the present instance, a strictly parallel series of control cultures in light was not maintained. But, in our experience, comparably high rates of mortality are not unprecedented in experimental cultures of *B. truncatus*, even under ordinary laboratory conditions ; they are therefore not necessarily connected with the absence of light (see below).

Two of the survivors reached sexual maturity at the beginning of March, the other two not until late April.

Copulation was not observed, but egg-masses appeared on the walls of the tank on 3.3.57, thus completing the life cycle of the snail from egg to egg in total and continuous darkness in 70 days.

Oviposition continued to take place, and by 30th March there were 26 egg-clutches, containing a total of 196 eggs. All of these eggs hatched between the 7th and 31st March.

On the 1st April, 25 infant snails of the second generation were transferred to separate tanks under laboratory conditions in the light for other investigations, leaving only 171 snails in the incubator tank in darkness. Of these 171, only two eventually survived beyond the end of April, together with a single individual from the first generation. However, since all of the infant snails transferred to other tanks in the light died within a period of 22 days, it is evident that the high juvenile mortality rate was not necessarily connected with darkness in this case.

The three survivors in the experimental dark tank throve until 11.5.57 when the experimenters, for reasons beyond their control, were obliged suddenly to leave the laboratory unattended. On their return, 20 days later, they found the three snails dead. Their death, of course, might have been due to many factors such as shortage of food supply, continuous stagnancy and increasing auto-pollution of the water and heavy decay of organic matter.

Discussion

It is interesting to compare our experiments with those of Deschiens and Bijan (1956) and Deschiens (1957).

Our aquaria contained 1·5 litres of water for a maximum snail population at any one time of 196 juveniles; whereas those of Deschiens contained 2 litres of water for a maximum snail population at any one time of 200 adults. Our aquaria were not aerated although the water was partially changed (500 ml.) at first twice a week and later daily; they were maintained at 25°C. to 27°C., and were supplied with strained spinach by way of food; whereas those of Deschiens were aerated at 3 litres of air per minute daily for 30 minutes, the water in them was not changed, temperature was maintained at 20°C. to 24°C., and lettuce leaves together with calcium phosphate and powdered fish were supplied as food. In both our experiments and those of Deschiens microscopic green algae were absent from the aquaria throughout the experiment.

Deschiens started his cycle with adult snails whereas we started ours with eggs.

There were remarkable differences in the findings. Whereas Deschiens observed oviposition to be rare and that the number of eggs per clutch was reduced to two or three, we found that it took place with normal frequency and that the number of eggs per clutch was the usual 7 or 8. Deschiens found that 50% of the eggs failed to develop and hatch ; whereas we found that normal hatching occurred in 100% of the eggs. In respect of juvenile mortality both sets of experiments showed a rate greater than 90%, but whereas in in the case of Deschiens such a juvenile death-rate was not observed in the snails in the control tanks in the light, in our case the juvenile death-rate, although high, was slightly lower in the dark tanks than in the light ones. It is only fair to remark, however, that on other occasions tanks in the light have shown much lower juvenile snail mortality rates in our laboratory. In both sets of experiments those juveniles which *did* develop reached maturity.

Deschiens was fortunate in being able to carry on his experiments for a longer period of time (365 days) than the authors (151 days). He was therefore able to observe that not only can a population of *Bulinus truncatus* cultured in darkness maintain its level for a year, but also that, albeit slowly and with difficulty, a gradual adaptation of the population to darkness is achieved.

Opinions have differed as to the relative importance of direct and indirect effects of light upon the snails. Our experiments clearly show that darkness does not lead to lowering of reproductive activity associated with absence of a direct stimulant effect of light on the endocrine and sexual glands. With regard to the indirect effects of darkness, these are chiefly manifest in the failure of green algae and water plants to grow in the absence of the light necessary for photosynthesis and the consequent deprivation of the snails of an important source of food. However, in the type of habit in which *B. truncatus* has actually been found breeding in darkness in nature, an ample supply of food in the form of fine vegetable debris and decaying organic matter, represented in our experiments by the strained spinach, is always present.

Concerning the high mortality rate of the juvenile snails in the dark tanks, so little is yet known about the mortality rate of juvenile snails in natural environments that it is not safe to deduce more than that population levels might be somewhat lower in dark conditions, other things being equal, than they would be in the light.

Conclusions

Although *B. truncatus* populations can become adapted to live and breed in total and continuous darkness, and have been so found in nature, absence of light has been shown both by our experiments and those of Deschiens to have a restrictive effect on their multiplication manifested chiefly by high juvenile mortality.

The use of pipes and covered channels for irrigation water, although not a complete protective measure, is therefore to be strongly recommended in schemes of control directed against the vectors of schistosomisasis haematobia ; particularly since it has the added advantage of preventing access by infected persons to snail-infested water. Maintenance of a continuous rapid flow will render the habitat still less favourable. Any breeding foci which should develop can then be relatively simply eradicated with molluscicides.

Two points should not be overlooked—in the first place that a single surviving individual of a hermaphrodite species such as *B. truncatus* can repopulate a habitat under favourable conditions ; and in the second place that planorbid snails have been shown by Deschiens and Bijan (1956) to be less susceptible to the restrictive effects of darkness than *B. truncatus*.

Summary

Starting with eggs from a natural habitat the authors were able to raise and breed *Bulinus truncatus*, the snail vector of schistosomiasis haematobia, under conditions of total and continuous darkness and to complete the life-cycle from egg to egg in 70 days. The rearing of a second generation was interrupted by circumstances beyond the authors' control. Darkness is shown nevertheless to have a restrictive effect on population growth and the use of covered channels and pipes for irrigation water, although not a complete control measure, should therefore be adopted wherever possible in endemic foci of schistosomiasis haematobia where snail control is necessary.

References

Barlow, C. H., 1937.—"The value of canal clearance in the control of schistosomiasis in Egypt." *Amer. J. Hyg.*, **22**, 376–391. (W.L. 600a.)

————, Abdel Azim, M. *et al.*, 1942–51.—First ten Annual Reports of the Bilharzia Snail Destruction Section of the Ministry of Public Health, Cairo. Government Press.

DESCHIENS, R., 1957.—"Sur la perpétuation des élevages des mollusques vecteurs des bilharzioses à l'obscurité." *Bull. Soc. Path. exot.*, **50**, 229–233. (W.L. 5310.)

————, and BIJAN, H., 1956.—"Comportement d'élevages de mollusques vecteurs des bilharzioses à l'obscurité." *Ibidem*, **49**, 658–661.

MARILL, F. G., 1943.—"Recherche des mollusques fluviatiles autour de grands barrages d'Algérie." *Arch. Inst. Pasteur Algér.*, **21**, 65–82. (W.L. 1842c.)

WATSON, J. M., 1958.—"Ecology and distribution of *Bulinus truncatus* in the Middle East. With comments on the effect of some human activities in their relationship to the snail host on the incidence of bilharziasis haematobia in the Middle East and Africa." *Bull. World Hlth. Org.*, **18**, 833–894. (French summary pp.891–892.) (W.L. 5748c.)

ZAKARIA, H., 1955.—"Further study on the ecology of the intermediate host of *Schistosoma haematobium*, *Bulinus truncatus* Baylis." *Bull. Endemic Dis. Baghdad*, **1**, 123–155.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 205–216.

A Study of the Gastrodiscinae Monticelli, 1892 (Gastrodiscidae : Paramphistomoidea)

By Sheila Willmott
Commonwealth Bureau of Helminthology
and
F. R. N. Pester
London School of Hygiene and Tropical Medicine

The original purpose of this paper was to present a short account of some aspects of the histology of the three genera of the subfamily Gastrodiscinae (Gastrodiscidae : Paramphistomoidea), namely, *Gastrodiscus* Leuckart, 1877, *Homalogaster* Poirier, 1883 and *Gastrodiscoides* Leiper, 1913. However, certain of the observations made during the course of this study have necessitated the revision of the last-named genus and the authors are proud to record that in their investigation of *Gastrodiscoides* they have been privileged to have the active co-operation of Professor R. T. Leiper.

This study was prompted, in the first instance, by the receipt of some specimens of *Homalogaster* from a goat at Comilla, East Pakistan. These were sent to one of us (S.W.) by Dr. Z. Hassan of the Pakistan Animal Husbandry Research Institute, through the kind offices of Dr. E. L. Taylor, to both of whom the authors wish to express their thanks. These specimens unfortunately did not section well but comparison with material in the collection of the Institute of Zoology of the University of Neuchâtel, and with the type specimens of *H. philippinensis* Stiles & Goldberger, 1908, which were kindly lent by Dr. Allen McIntosh, lead to the conclusion that the specimens from Comilla are identical with *H. paloniae* Poirier, 1883. Mr. S. Prudhoe also made specimens available from the British Museum (Natural History). There seems to be little doubt that this is the only valid species of the genus known at present, *H. poirieri* Giard & Billet, 1892 and *H. philippinensis* Stiles & Goldberger, 1908 being synonymous with it. This was made clear by Railliet, Henry & Bauche (1914).

All three genera of the Gastrodiscinae are now shown to have marked papillae on the ventral surface and the histology of these and certain features in the male ducts in the three genera appear to present certain interesting features which have not previously been studied. It is the purpose of this paper to record a number of observations, some of which are perforce incomplete owing to the shortage of well preserved material suitable for histological study. It is greatly hoped that this work may stimulate interest in the curious features which are found in this group, as in others, of the Paramphistomoidea.

Gastrodiscus

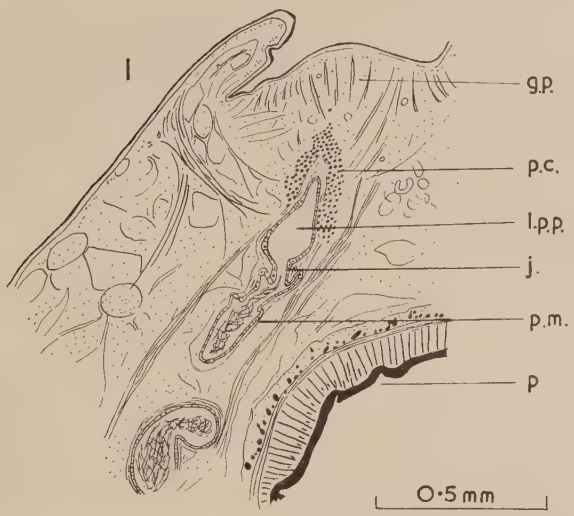
Specimens of both species of this genus, namely, *Gastrodiscus aegyptiacus* (Cobbold, 1876) and *G. secundus* Looss, 1907, were examined, material being made available to the authors from the collection in the London School of Hygiene and Tropical Medicine and the Liverpool School of Tropical Medicine, and also by Dr. W. E. Ormerod of the London School of Hygiene and Tropical Medicine and Mr. W. M. Fitzsimmons of the Commonwealth Bureau of Helminthology, St. Albans.

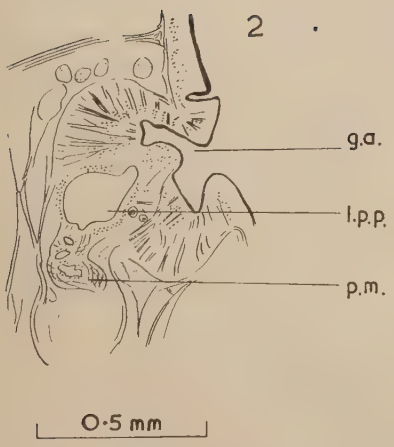
Male Ducts (Figs. 1, 2, 3)

In neither of the two species were cilia observed throughout the whole length of the vas deferens, in contrast to our findings in both *Homalogaster* and *Gastrodiscoides*. There is a longish pars musculosa which opens into the "pars prostatica" through a narrow neck-like region; this is muscular and protrudes into the "pars prostatica" slightly but there is no ciliated valve and the "pars prostatica" is not dilated as in the two other genera of the subfamily. There is no ciliated lining but the epithelial cells are much flattened and have indistinct cell walls. The prostate cells are slightly better developed and more closely associated with the walls of the duct in *Gastrodiscus* but in none of these three genera is there a true pars prostatica. As in the two others this narrows suddenly into a ductus ejaculatorius which is lined with cuticle. There is no cirrus pouch as previously described by Looss (1896) and he was also of the opinion that prostatic cells were lacking.

Papillae (Fig. 5)

These were well described and illustrated by Looss (1896) in his original description of *G. aegyptiacus*. They are definitely





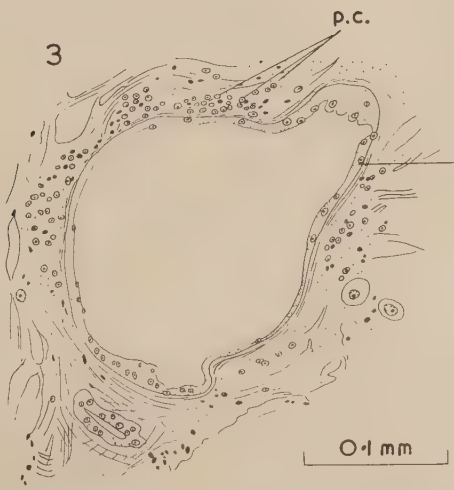


Fig. 1.—*Gastrodiscus aegyptiacus* male duct. Fig. 2.—*Gastrodiscus secundus* male duct. Fig. 3.—*Gastrodiscus secundus* male duct enlarged.

retractile and contain very obvious ducts. These were called excretory ducts by Looss in his original description but in his later paper (1912) he refers to the lymph system in *Gastrodiscus*, as in other paramphistomes. It was also pointed out by Willey (1930) that these are undoubtedly lymph canals. In the rest of the body wall these lymph canals appear very striking, being regularly arranged as circular and oblique ducts just within the outer wall. Yamaguti (1958) queries the lymph system in *Gastrodiscus* but there is no doubt that it is extremely well developed and especially clearly shown in the dorsal body wall. (Fig. 4).

In the papillae themselves there is very little musculature and it seems not unlikely that it is pressure within the body, possibly within the lymph system, which forces them to extrude. The presence of very prominent lymph canals within the papillae would also indicate that they may absorb nutritive materials from the surrounding medium or possibly play some part in extra-cellular digestion. It seems, in any case, improbable that they function as accessory suckers as they are so meagrely equipped with muscles. The fact that the cuticle over them is relatively very thin would support the view that they are absorptive. No nerve fibres could be demonstrated by either silver nitrate impregnation or by the lead haematoxylin staining technique.

Homalogaster paloniae

Specimens of this species have been examined from the following sources : the collection in the London School of Hygiene and Tropical Medicine (from cattle in Assam), the collection of the Institute of Zoology of the University of Neuchâtel (from cattle in Hardema), from a goat in East Pakistan and from the collection of the British Museum (Natural History) (from sheep in Hong Kong).

Male Ducts (Figs. 6, 7)

In this trematode the male ducts appear to be ciliated throughout almost their entire length, with the exception of the ejaculatory duct which is lined with cuticle. The vasa efferentia and the early part of the vas deferens are thin-walled but lined with ciliated epithelium. The more distal part of the vas deferens forms a distinct pars musculosa ; this passes into that part which has hitherto been termed "pars prostatica". At the junction of these two regions there is a definite ciliated valve-like structure similar

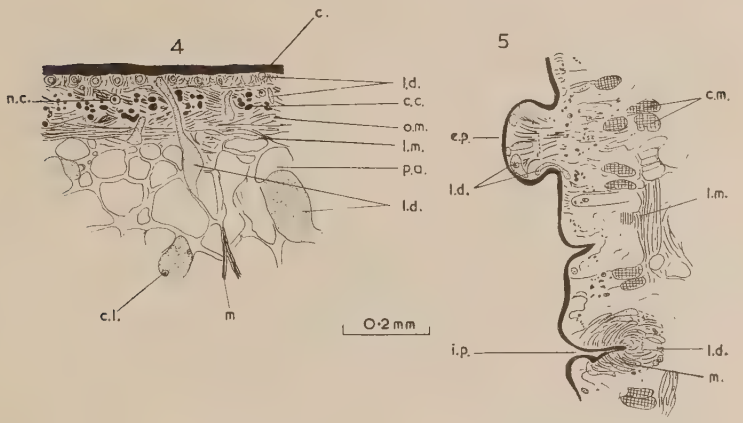


Fig. 4.—*Gastrodiscus aegyptiacus* dorsal body wall. Fig. 5.—*Gastrodiscus aegyptiacus* papillae.

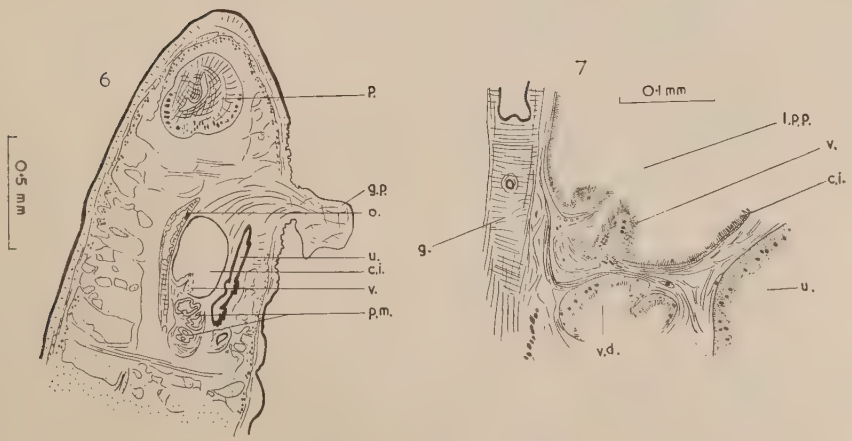


Fig. 6.—*Homalogaster paloniae* male duct. Fig. 7.—*Homalogaster paloniae* male duct showing ciliated lining.

to that previously described by Willmott (1960) in *Brumptia bicaudata*. Beyond this valve the duct is much dilated in all the specimens of which sections have been studied, irrespective of whether the genital papilla is extruded or no. This was noted by Stiles & Goldberger (1910). In section this structure appears to be almost spherical and is lined with ciliated epithelium. There seem to be no cells either in the wall, or associated with it, which could be termed prostate cells and Stiles & Goldberger, in their description remark "that were it not for analogy, one would probably hesitate to interpret them as prostatic cells". The term "pars prostatica" does not seem applicable in this species and we would now suggest that "ciliated chamber" would be more appropriate. This narrows abruptly at its distal end and becomes the ejaculatory duct which is, as stated earlier, lined with cuticle. The hermaphrodite duct seems to be extremely variable and its presence or absence depends apparently on the state of extrusion of the genital papilla. Maplestone (1923) correctly regarded this as of little diagnostic value.

Papillae (Figs. 8, 8a, 9, 10)

The gross appearance of the ventral papillae has been accurately described by Fukui (1929) who also made a study of their histology. He, however, regarded them as "mere evaginations of the body matrix" and could discern little structure in them. The present study, although able to demonstrate more detail than has been previously recorded has not been able to elucidate their complete structure and function. It is clear, nevertheless, that they are not retractile, in marked contrast to *Gastrodiscus*, although provided with their own circular and longitudinal muscles : each has a pit or depression in the centre and, in some sections, there are indications of minute pores opening at the base of this pit and leading into the, apparently, hollow interior of the papilla. This would hint at a possible glandular function, secreting either digestive enzymes or a protective covering for the parasite. Occasional nerve cells are visible but all attempts to demonstrate nerve fibres, and hence a sensory function, by means of silver nitrate impregnation or lead haematoxylin were entirely unsuccessful. The muscle layers of the ventral body wall under the papillae are not arranged in continuous sheets as in the dorsal body wall but rather in the form of a network with lymph ducts, parenchymatous and possibly glandular tissue passing through the meshes into each papilla. This mesh-like arrangement of the muscles on the ventral surface would account for the very typical curvature towards the dorsal side on fixation.

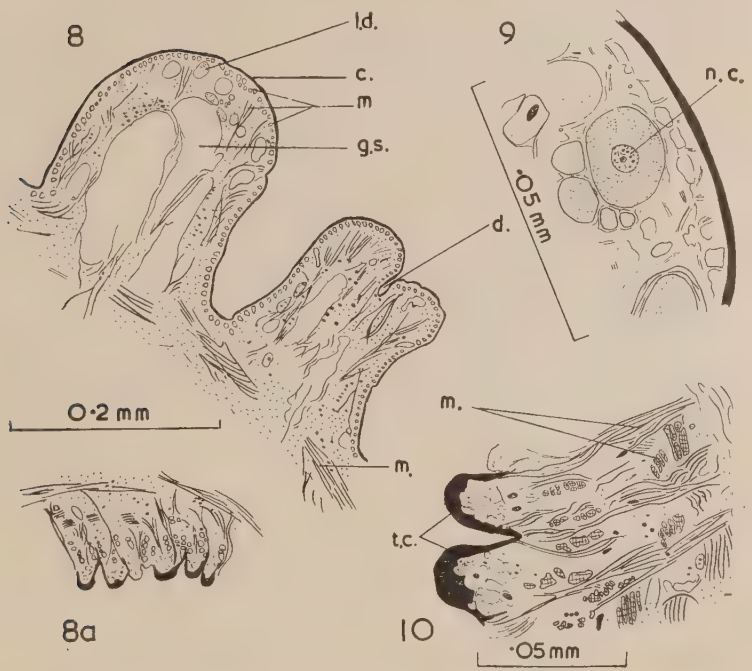


Fig. 8.—*Homalogaster paloniae* ventral papillae. Fig. 8a.—*Homalogaster paloniae* papillae around genital atrium (to same scale as Fig. 8). Fig. 9.—*Homalogaster paloniae* nerve cell in ventral papilla. Fig. 10.—*Homalogaster paloniae* papillae around genital atrium.

Abbreviations used in Figs. 1–13

c.=cuticle ; **c.c.**=cuticle-forming cells ; **ci.**=ciliated expanded portion of male duct ; **c.l.**=cells in lymph canal ; **c.m.**=circular muscle ; **d.**=depression in centre of papilla ; **e.**=unciliated epithelium ; **e.p.**=evaginated papilla ; **g.**=gut ; **g.a.**=genital atrium ; **g.p.**=genital papilla ; **g.s.**=?reservoir for glandular secretion ; **i.p.**=invaginated papilla ; **j.**=junction of pars musculosa and "pars prostatica"; **l.d.**=lymph ducts ; **l.m.**=longitudinal muscle ; **l.p.p.**=lumen of "pars prostatica"; **m.**=muscle ; **n.**=nuclei ; **n.c.**=nerve cell ; **o.**=oesophagus ; **o.m.**=oblique muscle ; **p.**=pharynx ; **pa.**=parenchyma ; **p.c.**=prostatic cells ; **p.m.**=pars musculosa ; **t.c.**=thickened cuticle ; **u.**=uterus ; **v.**=valve ; **v.d.**=vas deferens.

The papillae around the genital atrium are much smaller, conical, with rounded apices and covered with much thicker cuticle than are the ventral papillae ; they are altogether denser in appearance. They do not appear to be provided with circular muscles. (Figs. 8a, 10). Although the papillae around the mouth are different again in appearance, insufficient detail could be made out to warrant making any assertions as to structure or function.

Gastrodiscoides hominis

Specimens from several collections from man in the London School of Hygiene and Tropical Medicine were examined. Most of our observations, however, were made on a small collection of exceptionally well preserved material obtained from a Rhesus monkey and fixed by one of us (F.R.N.P.). This appears to be a new host record.* Additional observations were also made on Lewis & McConnell's type material of *Gastrodiscus hominis* (1876), Leiper's material of *Gastrodiscoides hominis* (1913), Khalil's specimens of *G. hominis* from the mouse deer (1923) and Varma's specimens of *G. hominis* var. *suis* (1954).

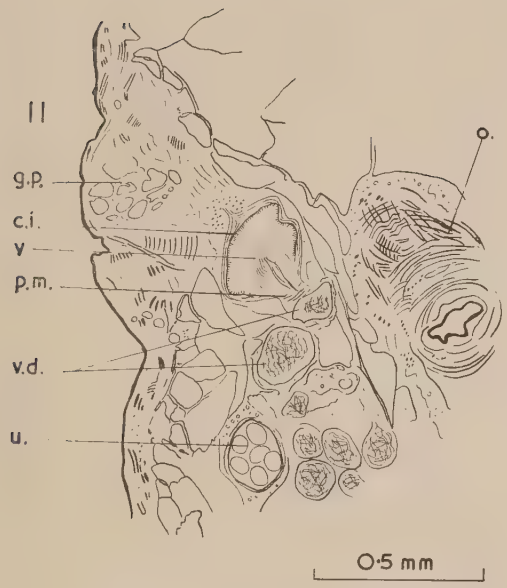
Male Ducts (Figs. 11, 12)

In this genus the male ducts are rather different from those in *Homalogaster* and *Gastrodiscus*. The main part of the vas deferens is not ciliated and this leads into a pars musculosa which also lacks a ciliated lining. This in turn opens into the so-called "pars prostatica" and at the junction there is a bulb-like, valvular projection similar to that in *Homalogaster* and *Brumptia*. The "pars prostatica" is again much dilated and this is lined with cilia although they are not as well developed as in *Homalogaster*. In *Gastrodiscoides hominis* there are a few scattered groups of cells associated with the vesicular portion which may be prostatic cells ; there is, however, no distinct pars prostatica. The ejaculatory duct, which leads from this part of the vas deferens to the exterior, is lined with cuticle.

Papillae (Fig. 13 and Plate 1)

None of the previous descriptions of this trematode have noted the presence of papillae on the ventral disc, in fact most have stated that they are definitely absent, although Stephens (1906) wrote "On the other hand, the cuticle over the ventral surface of the sucker is nowhere smooth as it is on the dorsum, but is

* Since this paper was submitted for publication *Gastrodiscoides hominis* has been recorded in a rhesus monkey by Graham, G. L., 1960, in *Ann. N.Y. Acad. Sci.*, **85** (3), 842–860. (W.L. 1051).



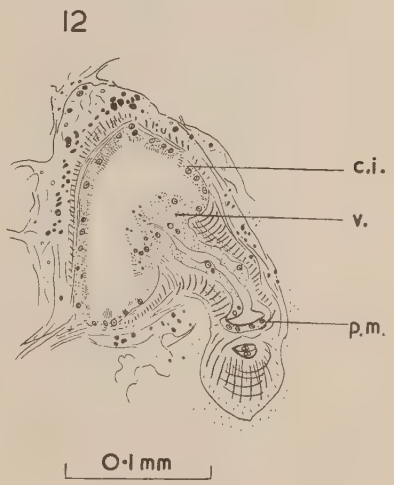


Fig. 11.—*Gastrodiscoides hominis* male duct. Fig. 12.—*Gastrodiscoides hominis* detail of valve.

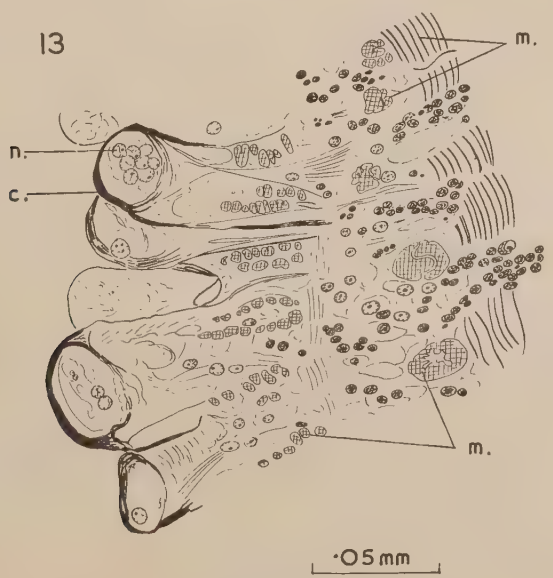


Fig. 13.—*Gastrodiscoides hominis* papillae

thrown into a number of microscopic ridges apparently caused by the disposition of the excretory tubules, which are very numerous in this position. These ridges are evidently not due to contractions of the cuticle for they are quite unlike artificial papillae produced by contractions elsewhere. They show no signs of being perforated when cut transversely to the surface". Leiper (1913) drew attention to the fact that Stephens used "sucker" here when he presumably meant the ventral disc.

Papillae are indisputably present on the surface of the ventral disc, although quite different in structure from those of *Gastrodiscus* and *Homalogaster*. They are not readily visible to the naked eye and are not retractile. In a thin hand section of the ventral disc, cut slightly obliquely to show the surface, they have a fairly regular "crazy pavement" appearance. In sagittal section they appear as definite, apparently almost hollow structures with groups of very large nuclei at the tip ; as many as nine of these nuclei have been observed in a single papilla ; there are no obvious cell walls surrounding them and they resemble the nuclei of the activo-germinal layer of mammalian tongue. There is little or no musculature within the papillae, only one or two irregular strands being apparent in some. What their function may be remains obscure. As far as we are able to judge there is no particular accumulation of excretory ducts in this region, although Stephens (1906) considered that there was.

It is worthy of note that these papillae are present in all the material examined and, in fact, that they are shown quite well in one of the photomicrographs used by Varma to illustrate his paper in 1954.

Discussion

The most notable point in these observations is that papillae are present in *Gastrodiscoides*. Lewis & McConnell (1876) make no mention of them ; Stephens (1906) refers only to cuticular ridges ; Leiper (1913) uses the absence of papillae as one of the points of distinction between *Gastrodiscus* and *Gastrodiscoides* ; Khalil (1923) states "The discoidal portion of the worm is devoid of papillae or pseudo-suckers, as seen in *Gastrodiscus* ; even Lane (1922, 1924) in the demonstration and paper in which he threw grave doubts on the validity of *Gastrodiscoides*, mainly because of his observation of a specimen with a genital atrium, did not apparently notice these papillae.

Leiper based his diagnosis on four main characters : (i) large genital cone ; (ii) position of genital orifice ; (iii) smooth ventral disc ; (iv) testes tandem. In our opinion Maplestone (1923) was

quite correct in his contention that the presence or absence of a genital atrium or genital cone or papilla was not significant in diagnosis, as it largely depended on the state of contraction of the worm. The ventral disc has, in the present paper, been shown to be provided with papillae. This leaves only the position of the genital orifice and the tandem arrangement of the testes remaining of the original four diagnostic characters. Nevertheless, two additional points of distinction have been added, namely the differences in the structure of the male genital ducts and the outstandingly different histology of the papillae, and we are of the opinion that *Gastrodiscoides* Leiper, 1913 is a valid genus.

Both the subfamily Gastrodiscinae Monticelli, 1892 and the genus *Gastrodiscoides* Leiper, 1913 are therefore redefined as follows : Gastrodiscinae (Gastrodiscidae : Paramphistomoidea) : body divided into two portions of which the anterior is small and the posterior dorso-ventrally flattened and covered with papillae ; ventral pouch and genital sucker absent ; acetabulum ventral at posterior end, small ; true pars prostatica and cirrus pouch lacking. *Gastrodiscoides* : with the characters of the subfamily ; genital opening at the level of the oesophageal bulb ; ventral disc not well defined, covered with non-retractile papillae ; testes tandem.

SUMMARY

All three genera of the Gastrodiscinae, namely, *Gastrodiscus* Leuckart, 1877, *Homalogaster* Poirier, 1883 and *Gastrodiscoides* Leiper, 1913 are shown to lack a true pars prostatica and cirrus pouch. The two last-mentioned have some part of the male ducts provided with cilia and all three possess papillae on the ventral surface ; these papillae are demonstrated to have a very different structure in each of the three genera. These and the male ducts are described and the subfamily Gastrodiscinae and the genus *Gastrodiscoides* are redefined.

Gastrodiscoides hominis is recorded from a Rhesus monkey.

ACKNOWLEDGMENTS

We should like to express our gratitude to all those who have given or lent us material, namely, Professor Jean G. Baer, Professor J. J. C. Buckley, Mr. W. M. Fitzsimmons, Dr. Z. Hassan, Professor W. E. Kershaw, Dr. Allen McIntosh, Dr. W. Ormerod, Mr. S. Prudhoe and Dr. E. L. Taylor. One of us (S.W.) wishes to thank Professor Baer for his hospitality and help in the Institut de Zoologie, Université de Neuchâtel, where this work was started and Professor Buckley for granting laboratory facilities in the Department of Parasitology,

London School of Hygiene and Tropical Medicine, where it was completed. Both of us are much indebted to Professor R. T. Leiper for his active assistance and invaluable advice and help with the literature.

Bibliography

Fischoeder, F., 1903.—"Die Paramphistomiden der Säugethiere." *Zool. Jb., Abt.* **1**, **17**, 485–660. (W.L. 23833.)

Fukui, T., 1909.—"Studies on Japanese amphistomatous parasites, with revision of the group." *Jap. J. Zool.*, **2**, 219–351. (W.L. 10881w.)

Giard et Billet, 1892.—"Sur quelques trematodes parasites des boeufs du Tonkin." *C.R. Soc. Biol., Paris*, 613–615. (W.L. 6630.)

Houdemer, E. F., 1938.—Recherches de parasitologie comparée Indochinoise. E. Le François, Paris. 235pp.

Khalil, M., 1923.—"A description of *Gastrodiscoides hominis* from the Napu Mouse Deer." *Proc. R. Soc. Med.*, **16**, 8–14. (W.L. 16916.)

Lane, C., 1922.—(Demonstration.) *Trans. R. Soc. trop. Med. Hyg.*, **16**, 22. (W.L. 21671.)

————, 1924.—"*Gastrodiscus hominis*." *Ann. trop. Med. Parasit.*, **18**, 27–32. (W.L. 1063.)

Leiper, R. T., 1913.—"Observations on certain helminths of man." *Trans. Soc. trop. Med. Hyg.*, **6**, 265–297.

Lewis, T. R. and McConnell, J. F. P., 1876.—"*Amphistoma hominis* n.sp., a new parasite affecting man." *Proc. Asiat. Soc. Beng.*, 182. (W.L. 16647.)

Looss, A., 1896.—"Recherches sur la faune parasitaire de l'Egypte." *Mém. Inst. égypt.* (*Égypte*), **3**, 1–252. (W.L. 13247.)

————, 1907.—"On some parasites in the museum of the School of Tropical Medicine. Liverpool." *Ann. trop. Med. Parasit.*, **1**, 121–154. (W.L. 1063.)

————, 1912.—"Über den Bau einiger anscheinend seltner Trematoden-Arten." *Zool. Jb.*, (*Suppl.* 15. *Festschrift J. W. Spengel*), **1**, 323–366. (W.L. 23835).

Maplestone, P. A., 1923.—"A revision of the Amphistomata of mammals." *Ann. trop. Med. Parasit.*, **17**, 113–208. (W.L. 1063.)

Poirier, J., 1883.—"Description d'helminthes nouveaux du *Palonia frontalis*." *Bull. Soc. philom. Paris*, (1882–83), **7**, 73–80. (W.L. 5324.)

Railliet, A., Henry, A. C. L. et Bauche, J., 1914.—"Sur les amphistomiens des ruminants domestiques de l'Annam." *Bull. Soc. cent., Méd. vét.*, **68**, 195–201. (W.L. 5020.)

Stephens, J. W. W., 1906.—"Note on the anatomy of *Gastrodiscus hominis*." *Thomp. Yates Labs. Rep.*, 7. (W.L. 21191.)

Stiles, C. W. and Goldberger, J., 1908.—"Observations on two new parasitic trematode worms : *Homalogaster philippinensis* n.sp., *Agamodistomum nanus* n.sp." *Bull. U.S. hyg. Lab.*, No. 40, 23–33. (W.L. 4295.)

————, 1910.—"A study of the anatomy of *Watsonius* (n.g.) *watsoni* of man and of nineteen allied species of mammalian trematode worms of the superfamily Paramphistomoidea." *Ibidem*, No. 60, 264pp.

Travassos, L., 1934.—"Synopse dos Paramphistomoidea." *Mem. Inst. Osw. Cruz*, **29**, 19–178. (W.L. 13465.)

Varma, A. K., 1954.—"Human and swine *Gastrodiscoides*." *Indian J. med. Res.*, **42**, 475–479. (W.L. 9940.)

Willey, C. H., 1930.—"Note on the interpretation and relations of lymph and excretory channels in the trematode *Hexangium sigani* Goto and Ozaki, 1929." *Trans. Amer. micr. Soc.*, **49**, 62–65. (W.L. 21400.)

Willmott, S., 1960.—"The morphology of *Brumptia bicaudata* (Poirier, 1908) Odhner, 1926 (Trematoda : Paramphistomoidea)." *Proc. zool. Soc. Lond.*, **134**, 623–634. (W.L. 16969b.)

Yamaguti, S., 1958.—Systema helminthum. Volume I. The digenetic trematodes of vertebrates. New York : Interscience Publishers, Inc., Part I, pp. xi+979 ; Part II, pp. 981–1575.

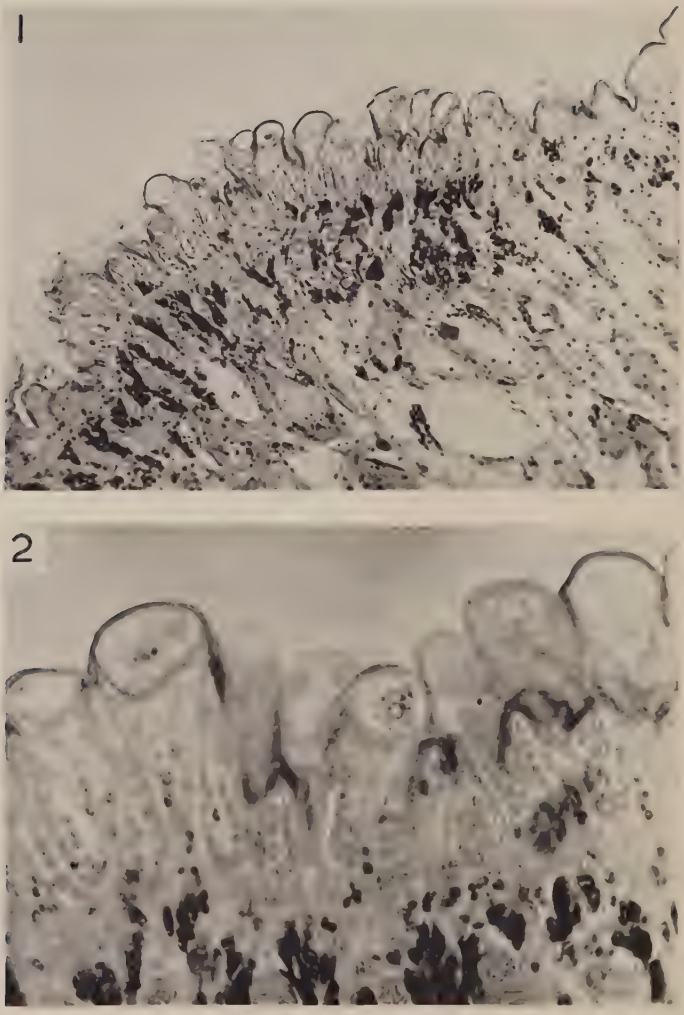


Plate 1.—*Gastrodiscoides hominis* papillae

Fig. 1.— × 300 approx. Fig. 2.— × 600 approx.

Journal of Helminthology, R. T. Leiper Supplement, 1961, *pp.* 217–228.

Studies on the Helminth Fauna of Japan. Part 57. Nematodes of Fishes, III

By Satyu Yamaguti

Zoological Institute, Kyoto University, Kyoto, Japan

PAGE

Heterocheilidae Railliet et Henry, 1915 218
1. *Raphidascaroides chilomycteri* n.sp. 218
2. *Contracaecum rectum* n.sp. 219
3. *Contracaecum hapalogenyos* n.sp. 222
4. *Contracaecum gracile* Yamaguti, 1935 222

Cucullanidae Cobbold, 1879 223
5. *Cucullanus filiformis* Yamaguti, 1935 223
6. *Cucullanus muraenesocis* n.sp. 223

Philometridae Baylis et Daubney, 1926 226
7. *Philometra cryptocentri* n.sp. 226
8. *Philometra spari* n.sp. 226
9. *Philometra zebrini* n.sp. 227

It is my greatest pleasure and honour to contribute a part of the series of my studies on the helminth fauna of Japan in honour of Professor R. T. Leiper, one of the most distinguished helminthologists in the world. The material on which the present paper is based was collected at the Tamano Marine Laboratory of Okayama University on the Inland Sea of Japan except for a new species of *Raphidascaroides* which was taken by Mr. T. Yamamoto at the Marine Biological Laboratory, Kyoto University, on the Pacific coast of Wakayama Prefecture. Acknowledgments are due to the staff of the Tamano Marine Laboratory and also to Mr. Yamamoto for their generous supply of the material.

Heterocheilidae Railliet et Henry, 1915

1. *Raphidascaroides chilomycteri* n.sp.
Pl. I, Figs. 1–4

Habitat : Stomach of *Chilomycterus affinis* Günther.

Material : One mature male and three mature or gravid females, collected by Mr. Torao Yamamoto.

Locality : Shirahama, Wakayama Prefecture.

Male. Body 83 mm. in length, tapering anteriorly with maximum breadth of about 1·6 mm. (1·7 mm. under cover glass pressure) at about one third the body length from posterior extremity. Cuticle with very fine transverse striations. No lateral flanges. Head 0·44 mm. long by 0·44 mm. broad ; each lip with lateral cuticular expansions overlapping each other over the interlabia ; dorsal lip with two papillae, depressed at its anterolateral corner to interlock with the dorsolateral prominence of subventral lip ; subventral lip with one papilla, two lobes of its pulp asymmetrical, the dorsolateral lobe projecting a little more forward than the ventrolateral. Interlabia nearly half as long as lips. Nerve ring apparently immediately anterior to excretory pore which lies at a distance of 1·5 mm. from the head end. Oesophagus 10·3 mm. in length, with maximum width of 0·49 mm. in front of its posterior end. Ventriculus 0·44 mm. in diameter ; ventricular appendix digitiform, blunt-pointed, 1·3×0·24 mm. Posterior extremity ventrally coiled ; tail conical, covered at its blunt tip with numerous rudimentary spines. Spicules alate, nearly equal, funnel-shaped at proximal end ; right spicule 3·63 mm. long, left one 3·57 mm. No gubernaculum. About 30 pairs of preanal papillae ; the anteriormost pair about 5 mm. anterior to anus ; 5 posteriormost pairs very small like postanals, of which the first lies at the level of the anal opening, the remaining 6 being arranged in the subventral field, though the last one lies a little more medially than the others.

Female. Body 72–106 mm. long by 1·0–2·2 mm. broad, tapering anteriorly in oesophageal region ; greater posterior part rolled up ventrally like that of male ; tail conical, 0·66–0·88 mm. long, with button-like tip covered with rudimentary spines. Head 0·38–0·5× 0·38–0·53 mm. ; lips similar to those of male. Excretory pore 1·4–1·5 mm. from head end. Oesophagus 8·2–11·9×0·33–0·66 mm., ventriculus 0·3–0·77 mm. in diameter ; ventricular appendix 1·1–2·0

mm. long, up to 0·22 mm. wide near its blunt posterior end. Vulva 24–32 mm. from anterior extremity, dividing body length in ratio of 1:2·0–2·4. Eggs subglobular, 62–70×54–68μ; contained ovum 2-segmented when deposited.

This species differs distinctly from the most closely related *R. diodonis* (Thwaite, 1927) Olsen, 1952, from *Diodon hystrix* of Ceylon not only in body length but also in spicular length and in the position of the vulva as shown in the following table.

	R. diodonis	*R. chilomycteri* n.sp.
Body { Male	38–52× 1·1 mm.	83× 1·7 mm.
Body { Female	45–62× 1·7 mm.	72–106× 1·0–2·2 mm.
Spicules	0·30–0·39 mm.	3·57–3·63 mm.
Vulva dividing body length in ratio of	1 : 2·8–3·1	1 : 2·0–2·4

2. *Contracaecum rectum* n.sp.

Pl. I, Figs. 5–6

Habitat: Small intestine of *Hapalogenys* sp.

Material: A single mature male and fragments of immature males.

Locality and date: Inland Sea; September 9, 1957.

Unless otherwise stated, the following description is based on the type male specimen alone. Body 39 mm. long by 0·66 mm., with its posterior extremity not curved ventrally in contrast with other known members of the genus. Cuticle very finely striated transversely; a narrow but distinct flange extending along each side of body from behind head to posterior extremity, about 50μ wide at level of nerve ring. Head 0·17×0·23 mm.; tail conical, 0·13 mm. long, with its pointed tip covered ventrally with extremely minute spines probably representing rudiments of larval spines. Each lip has two papillae; interlabia about 60μ long. Nerve ring 0·72 mm. from head end; excretory pore opening just behind it. No cervical papillae. Oesophagus 4·62 mm. long by 0·23 mm. broad; ventriculus glandular, well constricted off from oesophagus, 0·22×0·17 mm.; ventricular appendix 1·39 mm. long by 0·11 mm. broad; intestinal caecum 2·06 mm. long.

Testis beginning a short distance posterior to ventricular appendix; vesicula seminalis very wide, about 9.35 mm. long; with nodular appendix projecting into ejaculatory duct. Latter duct

2·86 mm. long, 0·31 mm. wide at anterior end. There are 20 pairs (25 and 26 pairs in two paratypes) of preanal and 6 pairs of postanal, papillae, of which the posterior preanals and postanals are very small, whereas most of the preanals are large and very prominent, the anteriormost lying at the level of the anterior end of the ejaculatory duct ; of the five postanals one is anterior, another is lateral, and the other four are close to one another at about the middle of the tail. In addition to these paired papillae there is another, large, unpaired, median papilla immediately in front of the anus. Spicules slender, alate, nearly equal, about 4·4 mm. long (2·7–3·0 mm. in immature specimens).

As shown in the following table this new species resembles *Contracaecum coiliae* Yamaguti, 1941, so closely that a definite separation is not possible until further anatomical details are worked out on sufficient material. It is, therefore, regarded as provisional. The specific name refers to the straight posterior extremity.

	Contracaecum coiliae	*Contracaecum rectum* n.sp.
Body	26 × 0·62 mm.	39 × 0·66 mm.
Head	0·09 × 0·105 mm.	0·17 × 0·23 mm.
Tail	0·1 mm.	0·13 mm.
Oesophagus	2·75 × 0·16 mm.	4·62 × 0·23 mm.
Intestinal caecum	1·9 mm.	2·06 mm.
Ventricular appendix	1·2 × 0·1 mm.	1·39 × 0·11 mm.
Preanal papillae	29 pairs	20–26 pairs
Postanal papillae	6 pairs	6 pairs
Spicules	3·3–3·7 mm.	2·7–4·4 mm.
Host	*Coilia mystus*	*Hapalogenys* sp.
Locality	Sea of Ariake	Inland Sea

PLATE I

Raphidascaroides chilomycteri n.sp.

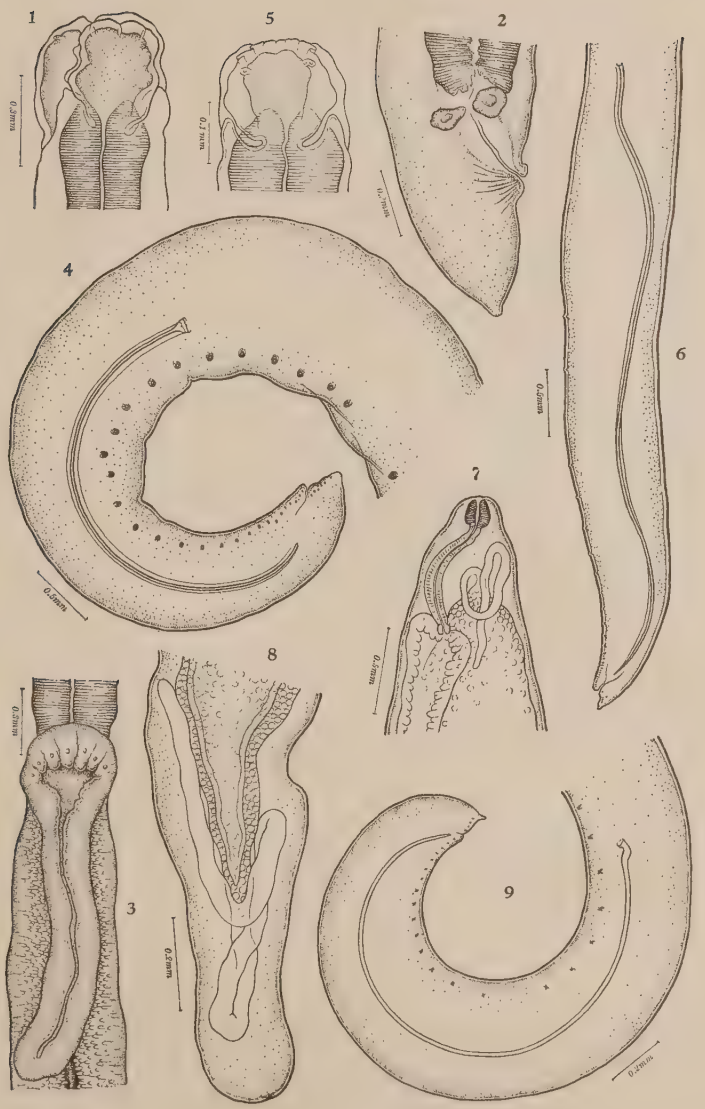
Fig. 1.—Anterior extremity of male, lateral view. Fig. 2.—Posterior extremity of female, lateral view. Fig. 3.—Ventricular region showing oesophagus, ventriculus and its appendix, and intestine, ventral view. Fig. 4.—Posterior extremity of male, lateral view.

Contracaecum rectum n.sp.

Fig. 5.—Anterior extremity of male, lateral view. Fig. 6.—Posterior extremity of male, lateral view.

Philometra cryptocentri n.sp., female

Fig. 7.—Anterior extremity, lateral view. Fig. 8.—Posterior extremity, ventral view. Fig. 9.—Posterior extremity of male of *Contracaecum hapalogenyos* n.sp., lateral view.


1
0.2mm
5
0.1mm
2
0.2mm
6
0.2mm
4
0.4mm
7
0.1mm
8
0.5mm
3
0.2mm
9
0.2mm

3. *Contracaecum hapalogenyos* n.sp.
Pl. I, Fig. 9 ; Pl. II, Fig. 10

Habitat : Small intestine of *Hapalogenys* sp.

Material : A single mature male and an immature female.

Locality and date : Inland Sea ; September 14, 1957.

Male. Body slender, filiform, 28×0·42 mm., tapering anteriorly and coiled ventrally at posterior extremity ; tail conical, 90μ long, with pointed tip covered with extremely fine spines. A narrow lateral flange extending from behind head to tail, 40μ wide in neck region. Head 88×133μ ; lips interlocking with each other at anterolateral corners ; interlabia triangular, 52μ long. Nerve ring 0·57 mm. from head end. Oesophagus 3·5 mm. long ; ventriculus 0·11×0·12 mm. ; intestinal caecum 2·37 mm. long, terminating 1·3 mm. from head end ; ventricular appendix 1·81×0·09 mm. Testis beginning just at level of posterior end of ventricular appendix. Seminal vesicle 4·95 mm. long, ejaculatory duct 2·4×0·22 mm. 22 pairs of preanal and 4 pairs of postanal, papillae ; first pair near base of spicule ; ventral transverse parietal musculature strongly developed in region of ejaculatory duct. Spicules slender, alate, about 2·0 mm. long.

Female (immature). Body 15·1×0·17 mm. ; tail 0·13 mm. long, terminating in a cone. Head 65×90μ ; nerve ring and excretory pore 0·37 mm. and 0·42 mm. respectively from head end ; oesophagus 1·78×0·08 mm. ; ventriculus 0·11×0·1 mm., intestinal caecum 1·2 mm. long, ventricular appendix 1·15×0·06 mm. Vulva 6·1 mm. from anterior extremity.

This species resembles *Contracaecum gracile* Yamaguti, 1935, very closely in general appearance, but differs from it in the spicules being shorter in spite of greater body length. It is to be noted that there is no unpaired median papilla immediately in front of the anus in the present species.

4. *Contracaecum gracile* Yamaguti, 1935

Three mature males found in the small intestine of *Conger myriaster* from the Inland Sea, together with my material reported from the same host species in 1941, will serve to define the limits of variation in measurements of this species as follows.

Male of *C. gracile* Yam., 1935 :

Body 19–27 × 0·27–0·44 mm., head 0·1–0·18 mm. broad ; tail 60–90μ long ; lips 0·09–0·15 mm. long ; interlabia 36–60μ long ; nerve ring 0·45–0·66 mm. from head end ; oesophagus 2·15–3·3 × 0·09–0·17 mm.; ventricular appendix 1·1–1·98 mm. long ; intestinal caecum 1·35–2·57 mm. long, terminating at a distance of 0·8–1·2 mm. from head end ; 18–34 pairs of preanal and 3 pairs of postanal, papillae, and unpaired median papilla immediately in front of anus. Spicules 2·0–3·7 mm. long.

CUCULLANIDAE Cobbold, 1879

5. *Cucullanus filiformis* Yamaguti, 1935

A number of mature males and females of this species from the small intestine of *Conger myriaster* from the Inland Sea gave the following measurements, which serve to supplement the specific diagnosis given in the original report of the species.

Male. Body 7·1–13·2 × 0·18–0·33 mm., tail 0·15–0·22 mm. long ; nerve ring 0·25–0·29 mm. from head end ; oesophagus 0·84–1·0 × 0·08–0·12 mm. ; spicules 0·8–1·1 mm. long ; gubernaculum 0·11–0·13 mm. long.

Female. Body 10·3–16·4 × 0·19–0·44 mm. ; tail 0·21–0·3 mm. long ; nerve ring 0·27–0·33 mm. from head end ; oesophagus 0·88–1·16 × 0·1–0·15 mm. Vulva dividing body length in ratio of 1·8–2·3:1. Eggs elliptical, 65–75 × 36–45μ.

As compared with the original measurements the above measurements are somewhat smaller except for the egg size. This is obviously due to difference in age of the specimens.

6. *Cucullanus muraenesocis* n.sp.

Pl. II, Figs. 11–12

Habitat : Small intestine of *Muraenesox cinereus* (Forskål).

Material : A single mature male.

Locality and date : Inland Sea ; April 28, 1957.

Body 9·13 mm. in length, with nearly uniform width of 0·27 mm. except for tail, which is 0·27 mm. long and tapers gradually to a sharp point. Anterior extremity bent dorsally at level of nerve ring. Head broadly rounded, only slightly wider than neck. Nerve ring 0·66 mm. from head end ; excretory pore just behind nerve ring ; cervical papillae 0·42 mm. from posterior end of oesophagus. Each lip with smooth cuticular border and three (one lateral and two submedian) head papillae. False buccal capsule 0·24 mm. in dorsoventral diameter, with small pad-like chitinous thickenings anteriorly. Oesophagus 1·35 mm. long, 0·23 mm. wide at basal swelling. There is no intestinal caecum, although the left wall of the intestine extends forward over the posterior end of the oesophagus in form of a dome. The posterior end of the intestine surrounded by a sphincter muscle projects into the rectum (cloaca) in form of a pointed cone.

Testis originating at a distance of 1·8 mm. from tail end, turning back on itself 0·85 mm. behind oesophagus. Preanal sucker reduced to a mere aggregate of several (9) muscle bundles arranged in one layer close to one another. The ductus ejaculatorius lined with cylindrical epithelia is only 0·2 mm. long and 56μ wide at its elliptical anterior swelling ; it opens into the cloaca ventral to the base of the above-mentioned intestinal cone. There are 11 pairs of anal papillae, of which three are preanal, two adanal and six postanal ; the first preanal lies just at the level of the rudimentary sucker, the second 0·27 mm. behind the first, the third 0·16 mm. behind the second, and the fourth (first adanal) 0·22 mm. behind the third. Of the six postanal papillae the first, fourth and sixth are submedian, but the second, third and fifth are lateral. Immediately in front of the cloacal aperture is an unpaired median papilla containing terminally enlarged pulp. Spicules hollow, slender, about 0·43 mm. long ; gubernaculum a simple rod 0·14 mm. long.

PLATE II

Fig. 10.—Anterior extremity of male of *Contracaecum hapalogenyos* n.sp., dorsal view.

Cucullanus muraenesocis n.sp.

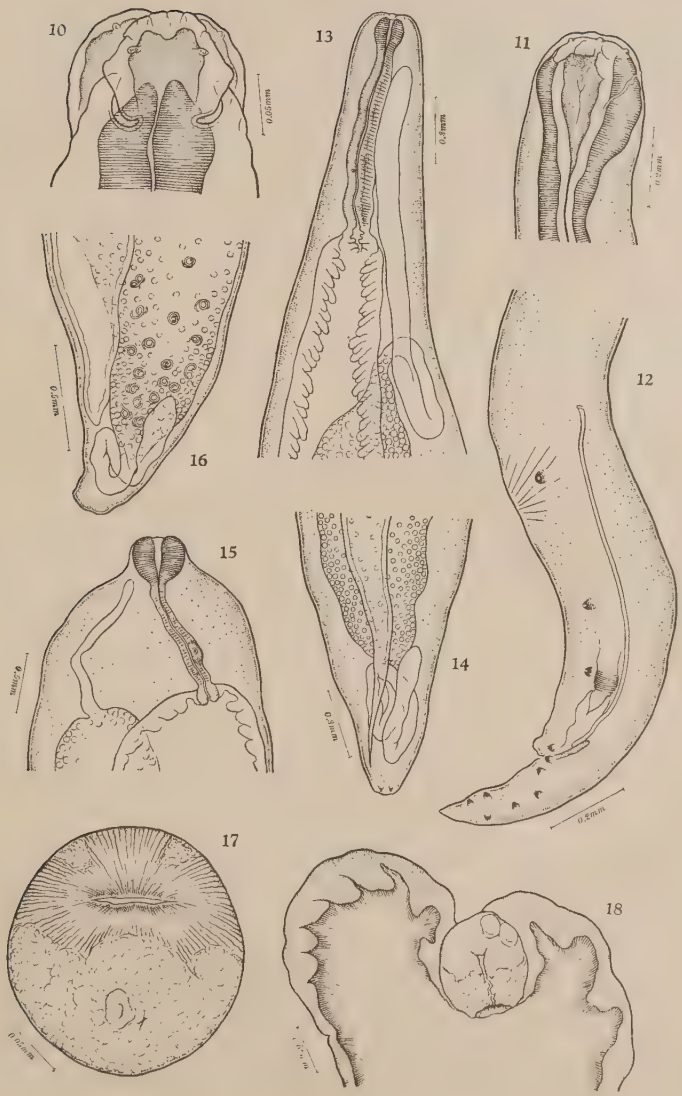
Fig. 11.—Anterior extremity of male, lateral view. Fig. 12.—Posterior extremity of male, lateral view.

Philometra spari n.sp., female

Fig. 13.—Anterior extremity, lateral view. Fig. 14.—Posterior extremity, ventrolateral view.

Philometra zebrini n.sp., female

Fig. 15.—Anterior extremity, lateral view. Fig. 16.—Posterior extremity, lateral view. Fig. 17.—Transverse section of oesophagus at level of its swollen part showing unpaired dorsal and paired subventral oesophageal glands. Fig. 18.—Transverse section at oesophago-intestinal junction.


10
0.05mm
13
0.3mm
11
0.1mm
16
0.5mm
12
15
0.1mm
14
0.05mm
0.2mm
17
0.05mm
18
0.1mm

Although the female is unknown, the male is characterized by the rudimentary preanal sucker and the arrangement of the anal papillae. In the known members of the genus the anteriormost preanal papilla usually lies in front of the preanal sucker and not in the sucker area.

Philometridae Baylis et Daubney, 1926

7. *Philometra cryptocentri* n.sp.
Pl. I, Figs. 7–8

Habitat : Body cavity of *Cryptocentrus filifer* (Cuv. et Valenc.).

Material : A single fully gravid female.

Locality and date : Inland Sea ; September 10, 1957.

Body filiform, 93 mm. long by 0·8 mm. wide, tapering at two extremities. Cuticle very thin and smooth. Head blunt-pointed, without papillae. Oesophagus 0·7 mm. long ; its anterior swelling 78μ wide, remaining portion cylindrical ; ventriculus about 50×80μ, followed by valvular appendage. Intestine very much widened at the commencement, tapered and obliterated posteriorly. Anterior ovary originating near anterior end of intestine, describing a double loop and opening into uterus 0·45 mm. from head end. Posterior ovary arising 0·8 mm. from blunt posterior extremity, where it forms an S-shaped curve before opening into the uterus. Embryos very slender, 0·28–0·35 mm. long by 10μ wide.

8. *Philometra spari* n.sp.
Pl. II, Figs. 13–14

Habitat : Swim bladder of *Sparus macrocephalus* (Basilewsky).

Material : A single not fully mature female.

Locality and date : Inland Sea ; September 9, 1957.

Body 110 mm. in length, with maximum width of 1·4 mm. near anterior extremity, tapering toward both extremities. Cuticle with very fine transverse striations. Head broadly rounded, without papillae. Oesophagus 0·83 mm. long, with a slender dorsal oesophageal gland extending throughout its length and apparently

continued onto ventricular portion which is inserted between the oesophagus proper and the intestine ; bulbous anterior swelling of oesophagus 0·12 mm. in diameter. Ventriculus 65μ long by 0·11 mm. wide, composed of glandular cells filled with granules, followed by trivalved appendage projecting into intestinal lumen. Intestine tapering posteriorly and apparently obliterated, without anus. Tail end truncate at tip, provided terminally with four inconspicuous papillae. Anterior ovary originating at a distance of 0·22 mm. from head end, turned back on itself before opening into uterus, which arises 1·5 mm. behind the head end and is filled with rounded immature ova of different dimensions (13–26μ). Posterior ovary forming two loops at posterior extremity.

This species is distinguished from any of the known members of the genus by the uterus arising behind the anterior end of the intestine. That it was found in the swim bladder is also worth noting.

9. *Philometra zebrini* n.sp.

Pl. II, Figs. 15–18

Habitat : Subcutaneous connective tissue of *Zebrias zebrinus* Temm. et Schl.

Material : Three gravid females and three young females.

Locality and date : Inland Sea of Japan ; April 30, 1957.

Body cylindrical, robust, blood red in life, 30–35 mm. long by 1·5–2·0 mm. broad ; cuticle unarmed, transversely striated. Head end usually very prominent owing to the oesophageal bulb projecting forward. Posterior extremity may be two-lobed in full-grown individuals, but rather pointed in younger individuals. Anterior oesophageal bulb spherical, with triradiate lumen, 0·3–0·5 mm. in diameter ; oesophagus 0·1–0·16 mm. in diameter, slightly swollen in form of a spindle a little behind middle, where the lumen is flattened and displaced excentrically by the swollen dorsal oesophageal gland, the subventral glands being rather reduced at this level. The extreme posterior end of the oesophagus projecting into the lumen of the intestine consists of spongy, loose-meshed parenchyma suggestive of a rudimentary ventriculus. On the same section two small, sharply defined, vesicular structures are seen in the wall of the oesophagus. It seems likely that these structures represent the swollen ducts of the oesophageal glands, though they could not be

traced up to the swollen portion of the dorsal oesophageal gland which is shown in Fig. 17. At this level of the gland there is a narrow duct of irregular outline close to the nucleus, whereas in the subventral glands there is no definite duct to be seen. Intestine very wide throughout, ending blindly near posterior extremity.

Anterior ovary tubular, 0·05–0·08 mm. wide, extending nearly straight backward from beside oesophageal bulb to anterior end of uterus. Uterus commencing a little behind anterior end of intestine in young individuals, but reaching oesophageal bulb in fully gravid worms ; posterior ovary also tubular, 0·09–0·13 mm. wide, turning back on itself around posterior end of uterus. No vulva. Embryo about 0·41 mm. long by 0·017 mm. wide.

This species is characterized by its robust body and its habitat which is always limited to the subcutaneous connective tissue ventral to the abdominal cavity of the fish.

References

Thwaite, J. W., 1927.—"On a collection of nematodes from Ceylon." *Ann. trop. Med. Parasit.*, **21**, 225–244. (W.L. 1063).

Yamaguti, S., 1935.—"Studies on the helminth fauna of Japan. Part 9. Nematodes of fishes, I." *Jap. J. Zool.*, **6**, 337–386. (W.L. 10881w).

————, 1941.—"Studies on the helminth fauna of Japan. Part 33. Nematodes of fishes, II." *Ibidem*, **9**, 343–396.

New Names in R. T. Leiper Supplement

New Subfamily

PAGE

ROBERTIINAE Travassos and Kloss, 1961 188

New Tribes

BRADYPOSTRONGYLEA Cameron and Myers, 1961 ... 30
GRAPHIDIOPSEA Cameron and Myers, 1961 32
MACIELIEA Cameron and Myers, 1961 28
SHATTUCKIEA Cameron and Myers, 1961 34
TRAVASSOSTRONGYLEA Cameron and Myers, 1961 ... 32

New Genera

HEPATICOFILARIA Ortlepp, 1961 136
ROBERTIA Travassos and Kloss, 1961 188

New Species

CHEVREUXIA LEIPERI Singh and Ali, 1961 159
CONTRACAECUM HAPALOGENYOS Yamaguti, 1961... 222
CONTRACAECUM RECTUM Yamaguti, 1961 219
CUCULLANUS MURAENESOCIS Yamaguti, 1961 ... 223
HEPATICOFILARIA PACHYCEPHALUM Ortlepp, 1961 136
MELOIDOGYNE ARTIELLIA Franklin, 1961 85
PHILOMETRA CRYPTOCENTRI Yamaguti, 1961 ... 226
PHILOMETRA SPARI Yamaguti, 1961 226
PHILOMETRA ZEBRINI Yamaguti, 1961 227
RAPHIDASCAROIDES CHILOMYCTERI Yamaguti, 1961 218
ROBERTIA LEIPERI Travassos and Kloss, 1961... ... 188
RICTULARIA LEIPERI Ortlepp, 1961 131
RICTULARIA MYONACIS Ortlepp, 1961... 135
TRICHOSOMOIDES NASALIS Biocca and Aurizi, 1961 ... 5

This supplement is issued free of charge to subscribers to the *Journal of Helminthology*. It can be purchased (30/– post free) from the Secretary, London School of Hygiene and Tropical Medicine, Keppel Street, W.C.1.